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Experimentelle Beiträge zu einer Analyse der spezifischen Induktionsleistung heterogener Induktoren

von HANS ENGLÄNDER *und* ANNA GISELA JOHNEN[1]

Aus dem Zoologischen Institut der Universität Köln

EINLEITUNG

DURCH die Experimente Mangold's (1933) war bewiesen worden, daß das in einzelne Teile zerlegte Urdarmdach bei der Prüfung seiner Induktionsfähigkeit leistungsspezifische Unterschiede zeigt. Es hatte sich ergeben, daß das Muster der im Urdarmdach liegenden Induktionstendenzen im großen und ganzen den später im darüberliegenden Ektoderm realisierten, regional differenzierten Bezirken der Medullarplatte entspricht. Mindestens drei große Komplexe scheinen durch verschiedenes Unterlagerungsmaterial im Ektoderm realisiert zu werden. Die cranial gelegenen Abschnitte des Urdarmdaches induzieren Vorderkopf-, die mittleren Hinterkopf- und die mehr caudalen Teile Rumpfschwanzgebilde. Es kann daraus gefolgert werden, daß die Wirkung des 'Organisators' nicht vollkommen unspezifisch ist, sondern daß das Urdarmdach hinsichtlich seiner Induktionsleistung regional spezifische Unterschiede zeigt.

In Hinblick auf dieses Ergebnis war es bedeutungsvoll, daß auch mit heterogenen Induktoren, besonders mit Geweben adulter Tiere, prinzipiell ähnliche Ergebnisse erzielt werden konnten (Holtfreter, 1934; Chuang, 1939, 1940; Toivonen, 1940; Rotmann, 1942). Auch die heterogenen Induktoren ließen sich in Gewebe mit unterschiedlicher Leistungsspezifität einteilen. Mit diesem Induktionsmaterial, das jeder Zeit in beliebiger Menge beschafft werden konnte, waren dem Untersucher Induktoren in die Hand gegeben, die die Hoffnung aufkommen ließen, den oder die Induktionsstoffe zu analysieren. Unter der Voraussetzung, daß tatsächlich stoffliche Unterschiede die spezifische Leistung bedingen, wurde das Induktionsmaterial verschiedenen chemischen und physikalischen Behandlungsmethoden ausgesetzt (Chuang, 1939, 1940; Toivonen, 1949; Toivonen & Kuusi, 1948), um durch Abstraktion die chemische Natur der wirksamen Agenzien zu klären.

In der vorliegenden Untersuchung soll durch bestimmte Beinflussung der Spendertiere bzw. des Implantatmaterials versucht werden, die chemisch-physiologische Zusammensetzung des Induktionsmaterials zu verändern, um

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festzustellen, welchen Einfluß diese Manipulationen auf die Leistungsspezifität haben. Zu diesem Zweck wurde die Wirkung verschieden langer Alkoholfixierung der Implantate untersucht. Außerdem wurde die Bedeutung des Alters und des Ernährungszustandes des Spendertieres in bezug auf die leistungsspezifische Wirkung der Implantate geprüft.

Um eine mögliche Beteiligung der Ribonucleinsäure beim Induktionsvorgang festzustellen, wurden in einigen Serien die Implantate vor dem Einstecken mit dem Ferment Ribonuclease behandelt.

Als Implantate wurden die Nierenrinde und Leber (Engländer), Gl. Parotis und die Herzmuskulatur (Johnen) vom Meerschweinchen gewählt.

MATERIAL UND METHODE

Die Implantate wurden nach Fixierung mit 70%igem Äthylalkohol und nach Auswaschen in Holtfreter-Lösung ins Blastocoel früher Gastrulae von *Triturus vulgaris* und *Triturus helveticus* (Harrison-Stadium 11a) eingesteckt. Die Dauer der Alkoholbehandlung der Implantate sowie Alter und Ernährungszustand der Spendertiere sind in den Tabellen angegeben.

Die operierten Larven wurden in Glasschälchen auf Agar-Agarboden in verdünnter Holtfreter-Lösung mit Zusatz von Elkosine (1 gr/lt) kultiviert und nach 18–20 Tagen (Harrison-Stadium 42–43) in Bouin fixiert. Schnittdicke: 10 μ, Schnittfärbung: Haemalaun-Eosin.

Für die Behandlung der Implantate mit dem Ferment Ribonuclease stand das Präparat der Firma Armour & Co., Chicago, zur Verfügung.

Bei der histologischen Auswertung der Schnittserien wurden die Keime in folgende Gruppen eingeteilt:

1. Positive Fälle = alle Keime, die einen identifizierbaren Induktionseffekt aufweisen.
2. Negative Fälle = Keime, bei denen das Implantat Kontakt mit der Epidermis hat, aber ohne Induktionseffekt.

Beide Gruppen zusammen ergeben die verwertbaren Fälle. Ausgeschieden wurden die Fälle, bei denen das Implantat nachweisbar ist, aber ein Kontakt mit der Epidermis nicht festgestellt werden konnte oder bei denen die induzierten Organe in Verbindung mit den primären stehen und deshalb eine Entscheidung, ob Induktion oder Spaltung, nicht zu treffen ist.

Innerhalb der Induktionsgebilde wurden archencephale, deuterencephale und spinocaudale Komplexe unterschieden auf Grund der von Lehmann (1938) analysierten regionalen Gliederung der Medullarplatte. Induktionskomplexe archencephalen Charakters (A-Komplex) enthalten Tel- und Diencephalon mit oder ohne Epi- und Paraphyse, Augen mit oder ohne Linsen, Nasen, freie Linsen oder Lentoide, Augenpigment, Balancer und Epidermisvorwölbungen. Komplexe deuterencephalen Charakters (B-Komplex) werden gekennzeichnet durch Mes- und Rhombencephalon und Ohrblasen. Der spinocaudale Komplex (C/D-

Komplex) wird charakterisiert durch Neuralrohr, Chorda, Muskulatur, Flossensaum mit Mesenchym, Nierenkanälchen und Blutzellen.

Die prozentuale Berechnung der allgemeinen Induktionsrate geht aus von der Zahl der verwertbaren Fälle pro Serie. Die Prozentwerte für die Häufigkeit des A-, B- und C/D-Komplexes wurden von der Zahl der positiven Fälle pro Serie berechnet.

Die statistische Signifikanz der erhaltenen Ergebnisse wurde mit der X^2-Methode festgestellt (Goulden, 1952). Als signifikant wurde $P < 1\%$, als schwach signifikant P-Werte zwischen 5% und 1% angenommen.

EXPERIMENTELLER TEIL

I. Die Beeinflußbarkeit der Leistungsspezifität von Nierenrinde- und Leberimplantaten

von **HANS ENGLÄNDER**

A. *Die Induktionsleistung der Nierenrinde*

1. *Der Einfluß des Alters des Spendertieres*

Es wurde die Induktionsleistung der Nierenrinde von verschieden alten Tieren (10 Tage, 4 Wochen und 6 Monate) geprüft. Sämtliche Implantate wurden kurzfristig, 3–5 Stunden, in 70%igem Alkohol fixiert.

Bei der äußeren Betrachtung der Induktionsgebilde, ungefähr 18–20 Tage nach der Operation, konnte zwischen den drei Serien kein Unterschied festgestellt werden. In allen traten Buckel mit Schwänzen gehäuft auf. Auch die histologische Untersuchung ergab das gleiche Bild: Deuterencephale Organe und spinocaudale Gebilde wie Chorda, Neuralrohr und Muskulatur, überwogen in allen Serien (Tab. 1).

TABELLE 1

Induktionsleistung der Nierenrinde in Abhängigkeit vom Alter der Spendertiere (Ernährung der Spendertiere: normal, Alkoholbehandlung der Implantate: 3–5 Std.)

Serie	*Alter d. Spender*	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*A*	*B*	*C/D*
N 7/49	10 Tg.	26	22	85	1 4%	13 59%	19 85%
N 6/51	4 Wo.	48	46	96	1 2%	23 52%	44 95%
N 4/50	6 Mo.	54	50	93	2 4%	28 56%	44 88%

Auf Grund der erhaltenen Ergebnisse kann festgestellt werden, daß Nierenrinde des Meerschweinchens nach kurzfristiger Behandlung mit Alkohol ein Induktor mit überwiegend deuterencephal-spinocaudaler Induktionswirkung ist. Diese Spezifität ist unabhängig vom Alter des Spendertieres (Homogenität signifikant).

Serie N 7/49 : Serie N 6/51 A : nicht A $X^2 = 0{,}05$ $P > 80\%$
B : nicht B $X^2 = 0{,}49$ $P > 30\%$
C/D : nicht C/D $X^2 = 0{,}76$ $P > 30\%$
Induktionsrate $X^2 = 0{,}01$ $P > 95\%$

Serie N 7/49 + Serie N 6/51 : Serie N 4/50 A : nicht A $X^2 = 0{,}04$ $P > 80\%$
B : nicht B $X^2 = 0{,}11$ $P > 70\%$
C/D : nicht C/D $X^2 = 0{,}29$ $P > 50\%$
Induktionsrate $X^2 = 0{,}04$ $P > 80\%$

2. *Die Abhängigkeit der Induktionsleistung von der Dauer der Alkoholbehandlung der Implantate*

Bei der Prüfung der Induktionsleistung der Nierenrinde stellte es sich heraus, daß die von früheren Untersuchern für nicht wesentlich gehaltene Dauer der Fixierung in 70%igem Alkohol zu einer Änderung der Spezifität der induzierten Komplexe führen kann. Nach länger dauernder Alkoholbehandlung der Implantate (etwa 8 Tage) ergab schon die äußere Analyse der induzierten Gebilde,

TABELLE 2

Induktionsleistung der Nierenrinde in Abhängigkeit von der Dauer der Alkoholbehandlung der Implantate (Ernährung der Spendertiere: normal)

Serie	*Alkohol-behandl.*	*Alter d. Spender*	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*A*	*B*	*C/D*
N 7/49	3–5 Std.	10 Tg.	26	22	85	1 4%	13 59%	19 85%
N 4/49	10 Tg.	14 Tg.	34	30	88	5 17%	13 44%	21 70%
N 6/51	3–5 Std.	4 Wo.	48	46	96	1 2%	23 52%	44 95%
N 6/49	4 Tg.	4 Wo.	28	25	89	2 8%	14 56%	20 78%
N 5/50	8 Tg.	5 Wo.	28	26	93	4 15%	12 46%	16 61%
N 1/51	10 Tg.	6 Wo.	27	24	89	17 71%	17 71%	8 33%
N 4/50	3–5 Std.	6 Mo.	54	50	93	2 4%	28 56%	44 88%
N 7/50	5 Tg.	6 Mo.	24	21	87	7 33%	12 57%	12 57%
N 2/50	5 Tg.	6 Mo.	26	23	88	8 35%	13 52%	10 43%
N 2/50a	9 Tg.	6 Mo.	27	23	85	16 70%	7 30%	4 17%
N 3/50	13 Tg.	6 Mo.	41	36	88	33 92%	18 50%	4 11%
N 4/50a	20 Tg.	6 Mo.	39	34	87	29 85%	18 53%	5 15%

daß die induzierten Schwänze stark abgenommen haben. Es konnten überwiegend buckelige Vorwölbungen mit Hirnteilen festgestellt werden. Die histologische Auswertung zeigte, daß die Zahl der spinocaudalen Organe zu Gunsten des archencephalen Komplexes stark vermindert war. Die Auszählung der induzierten Organe und Organkomplexe ergab in den Serien, wo die Implantate lange Zeit in Alkohol gelegen hatten (20 Tage), eine fast vollkommene Umkehr der Leistungsspezifität (Tab. 2).

Als Beispiel sollen die Induktionsergebnisse der Serien N 4/50, 3–5 Std. Alkoholbehandlung, und N 4/50a, 20 Tg. Alkoholbehandlung, verglichen werden (Organe des gleichen Spendertieres, Tab. 2, Fig. 1). Die kurzfristig in Alkohol fixierten Implantate induzieren nur in 4% der positiven Fälle archencephale Organe, deuterencephale Organe wurden in 56%, spinocaudale Organe in 88% der Fälle induziert. Komplette Schwänze traten in großer Zahl (50%) auf. Nach 20-tägiger Alkoholbehandlung der Implantate traten archencephale Organe in 85%, deuterencephale in 53% und spinocaudale nur noch in 15% der Fälle auf. Innerhalb des archencephalen Komplexes waren besonders häufig gut ausgebildete Gehirnteile (65%) und Nasen (44%) vertreten. Komplette Schwänze konnten nur noch in 6% der Fälle nachgewiesen werden. Die Induktionsrate ist in beiden Serien ungefähr gleich (93% und 87%).

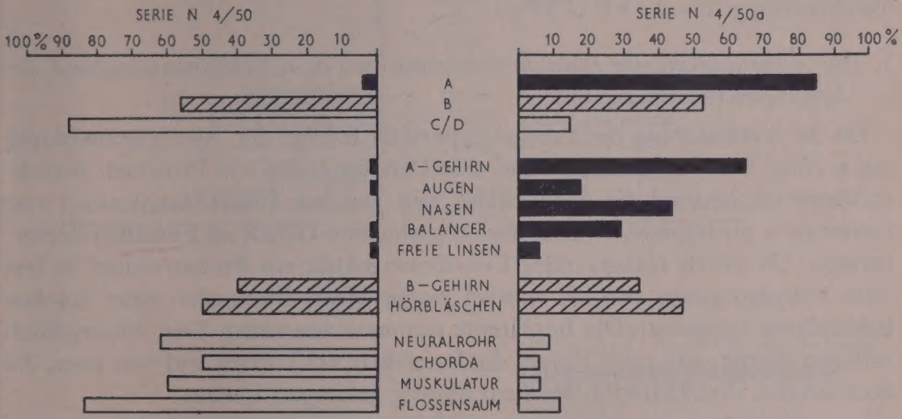

FIG. 1. Induktionsleistung der Nierenrinde vom Meerschweinchen nach kurzer (Serie N 4/50, 3–5 Std.) und nach langfristiger (Serie N 4/50a, 20 Tg.) Alkoholbehandlung der Implantate. Ernährung der Spendertiere: normal.

Ein Vergleich sämtlicher Serien der Alkoholreihen zeigt, daß die besprochene Änderung der Leistungsspezifität vom 5.– 8. Tag der Alkoholbehandlung deutlich in Erscheinung tritt. Allerdings ist der Effekt nicht in allen Serien gleich stark in Abhängigkeit von der Alkoholeinwirkungszeit, sondern es scheint, daß hier das Alter der Spendertiere eine Rolle spielt. Die Änderung der Spezifität ist am geringsten bei dem Induktionsmaterial, das von einem sehr jungen Tier

(14 Tg. alt) stammt (Serie N 4/49). Am deutlichsten tritt sie in Erscheinung bei Nierenrinde, die von Tieren stammt, die 6 Monate alt waren. Die Wirkung der Alkoholbehandlung wird hier schon nach 5 Tagen deutlich (Serie N 7/50 und N 2/50).

Zusammenfassend läßt sich sagen, daß es nach länger dauernder Alkoholbehandlung der Implantate zu einem Sinken der spinocaudalen Rate und zu einem Anstieg der archencephalen kommt. Die Induktionsfähigkeit für deuterencephale Organe wird nicht beeinflußt. Die Induktionsrate liegt in allen Serien ungefähr in der gleichen Höhe (Ergebnisse signifikant A : nicht A $X^2 = 183{,}59$ $P \leqslant 1\%$, B : nicht B $X^2 = 9{,}83$ $P > 30\%$, C/D : nicht C/D $X^2 = 67{,}85$ $P \leqslant 1\%$, Induktionsrate $X^2 = 2{,}43$ $P > 98\%$).

3. *Die Abhängigkeit der Induktionsleistung von dem Ernährungszustand der Spendertiere*

Da die Veränderung der Leistungsspezifität infolge der Alkoholeinwirkung mit größter Wahrscheinlichkeit auf eine Denaturierung von Proteinen zurückzuführen ist, bestand die Möglichkeit, den gleichen Effekt durch einen von vorneherein im Implantat vorhandenen geringeren Gehalt an Eiweißen hervorzurufen. Da durch Hunger oder Eiweißmangeldiät ein Proteinverlust in fast allen Körperorganen eintritt, wurden einige Meerschweinchen einer solchen Behandlung ausgesetzt. Die Implantate stammen von einem Tier, das reichlich gefüttert wurde, von zwei Tieren, die kurzfristig (1–2 Tage) und von zwei, die über längere Zeit (3 und 4 Wochen) hinweg gehungert hatten.

TABELLE 3

Induktionsleistung der Nierenrinde in Abhängigkeit von der Ernährung der Spendertiere (Alkoholbehandlung der Implantate: 3–5 Std.)

Serie	*Ernährung d. Spender*	*Alter d. Spender*	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*A*	*B*	*C/D*
N 6/51	normal	4 Wo.	48	46	96	1 2%	23 52%	44 95%
N 4/51	1 Tg. Hg.	6 Mo.	54	50	93	2 4%	28 56%	44 88%
N 3/51	2 Tg. Hg.	6 Wo.	67	63	94	11 18%	36 57%	49 78%
N 2/51	3 Wo. Hg.	6 Wo.	41	38	92	34 90%	19 50%	8 21%
N 5/51	4 Wo. Hg.	8 Wo.	55	53	96	48 90%	26 49%	12 23%

Die äußere Betrachtung der Induktionsgebilde zeigt, daß 1 Tag dauernder Hunger noch keinen sichtbaren Einfluß auf das Induktionsergebnis hat, es waren noch reichlich induzierte Schwänze vorhanden. Ihre Zahl verringerte sich aber schon in der Serie, in der die Implantate von einem Tier stammten, das zwei Tage gehungert hatte, und sie sind fast vollkommen verschwunden in den Serien, deren Induktionsmaterial von Tieren herrührte, die drei Wochen und länger nur ungenügend ernährt worden waren.

Die histologische Auswertung ergab (Tab. 3), daß ähnlich wie nach längerer

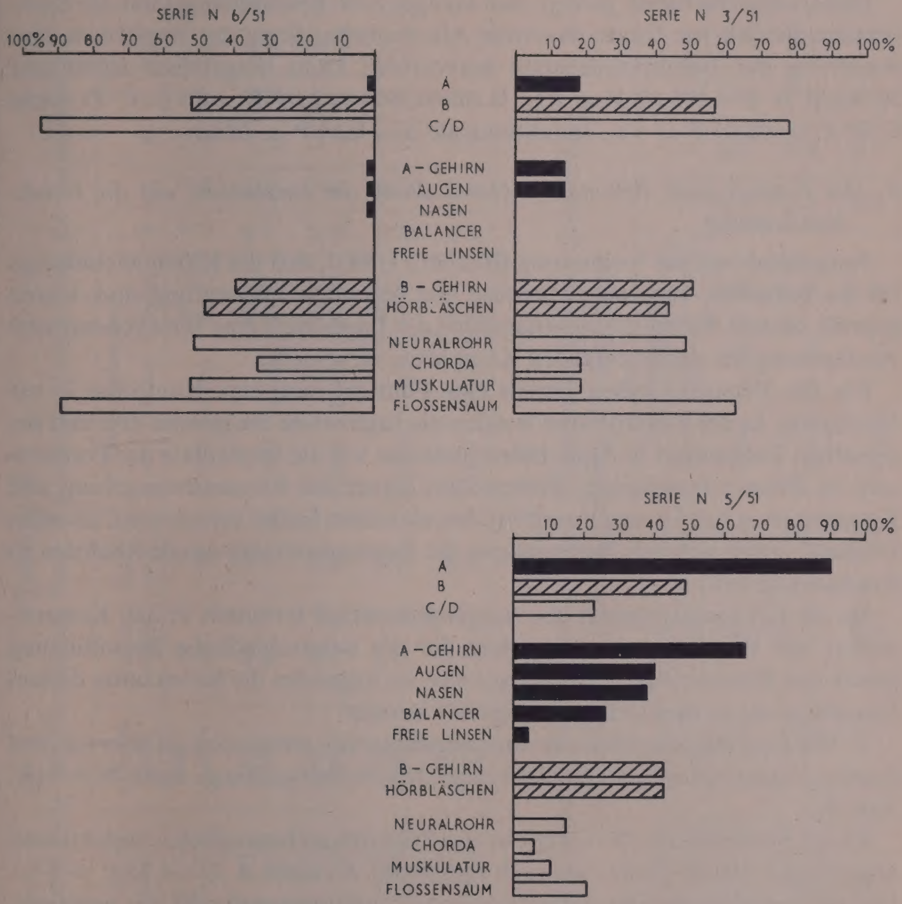

FIG. 2. Induktionsleistung der Nierenrinde vom Meerschweinchen bei normaler Ernährung (Serie N 6/51), nach kurzer (Serie N 3/51, 2 Tg. Hg.) und nach langer (Serie N 5/51, 4 Wo. Hg.) Hungerzeit der Spendertiere. Alkoholbehandlung der Implantate: 3–5 Std.

Alkoholbehandlung die spinocaudalen Organe zu Gunsten der archencephalen zurückgedrängt waren. Nach drei Wochen Hunger sind nur noch in 21% der Fälle spinocaudale Induktionsgebilde vorhanden, der archencephale Komplex ist auf 90% angestiegen. Die prozentuale Höhe der Zahl der deuterencephalen Organe verändert sich nur wenig.

Die Zahl der im einzelnen induzierten archencephalen Organe steigt in Abhängigkeit von der Dauer der Mangeldiät ziemlich gleichmäßig an. Bei den spinocaudalen Organen ist auffällig, daß Chorda und Muskulatur schon nach zwei Tagen Hunger ein sehr starkes Absinken aufweisen (Fig. 2).

Diese Versuche haben gezeigt, daß Hunger bzw. Eiweißmangeldiät der Spendertiere ähnlich wie länger dauernde Alkoholbehandlung der Implantate eine Änderung der Induktionsleistung hervorrufen kann (Ergebnisse signifikant A : nicht A $X^2 = 165{,}07$ $P \ll 1\%$, B : nicht B $X^2 = 1{,}09$ $P > 80\%$, C/D : nicht C/D $X^2 = 105{,}81$ $P \ll 1\%$, Induktionsrate $X^2 = 0{,}14$ $P > 70\%$).

4. *Der Einfluß einer Ribonucleasebehandlung der Implantate auf die Induktionsleistung*

Ausgehend von der Vermutung Brachet's (1942), daß die Ribonucleinsäuren für die Induktion von Neuralgeweben von besonderer Bedeutung sind, wurde geprüft, ob eine Ribonucleasebehandlung der Implantate eine Schwächung oder Auslöschung des archencephalen Komplexes verursacht.

Für die Versuche stehen jeweils eine Ferment- und eine Kontrollserie zur Verfügung. In der Kontrollserie wurden die Implantate die gleiche Zeit und bei derselben Temperatur in Aqua bidest. belassen wie die Implantate der Fermentserie in Ribonucleaselösung. Temperatur, Dauer der Fermentbehandlung und Konzentration der Lösung waren bei den einzelnen Serien verschieden, da nicht feststand, unter welchen Bedingungen die Fermentwirkung am deutlichsten in Erscheinung tritt.

Da die Leistungsspezifität des Ausgangsmaterials (ermittelt in den Kontrollserien) von Bedeutung zu sein schien für die unterschiedliche Beeinflussung durch eine Ribonucleasebehandlung sollen im folgenden die Serien unter diesem Gesichtspunkt in drei Gruppen eingeteilt werden:

1. Die Leistungsspezifität des Ausgangsmaterials erwies sich als überwiegend deuterencephal-spinocaudal (kurzfristige Alkoholbehandlung, Serie N 6/51K, Tab. 4).

In der Fermentserie (N 6/51F) ist die Zahl der archencephalen Induktionen angestiegen (Heterogenität schwach signifikant A : nicht A $X^2 = 4{,}83$ $P > 2\%$). Der spinocaudale und der deuterencephale Induktionsanteil sind nur unwesentlich verändert, ebenso die Induktionsaktivität (Heterogenität nicht signifikant).

2. Durch länger dauernde Alkoholbehandlung ist die spinocaudale Leistung des Ausgangsmaterials stark herabgesetzt, die archencephale erhöht (Serie N 3/50K, N 2/50K, N 1/50K und N 4/50K, Tab. 4).

Die Fermentbehandlung hat in dieser Versuchsgruppe praktisch keinen Einfluß auf die Spezifität des Induktors und dessen Induktionsaktivität (Serie N 3/50F, N 2/50F, N 1/50F und N 4/50F, Heterogenität nicht signifikant). Innerhalb des archencephalen Komplexes ließ sich eine mäßige qualitative Veränderung feststellen, die ihren Ausdruck in einem geringen Anstieg der Zahl der Gehirne und Nasen fand.

TABELLE 4

Induktionsleistung der Nierenrinde nach Ribonucleasebehandlung der Implantate

Serie	Alkohol-behandl.	verwertb. Fälle	positive Fälle(n)	Indukt.-rate (%)	Fermentbehandlung			A	%	B	%	C/D	%
					Konz.	Dauer	Temp.						
Kont. N 6/51K	3–5 Std.	48	46	96	..	..	20° C.	1	2	24	52	44	95
Ferm. N 6/51F	3–5 Std.	27	24	89	1:2500	4 Std.	20° C.	5	20	16	67	20	84
Kont. N 3/50K	8 Tg.	52	49	95	..	..	40° C.	42	86	30	61	2	4
Ferm. N 3/50F	8 Tg.	45	41	91	1:2500	2 Std.	40° C.	37	90	16	39	1	2
Kont. N 2/50K	9 Tg.	45	41	91	..	..	40° C.	31	75	14	34	5	12
Ferm. N 2/50F	9 Tg.	29	26	90	1:2500	2 Std.	40° C.	24	92	9	35	1	4
Kont. N 1/50K	12 Tg.	24	20	84	..	..	40° C.	12	60	12	60	5	22
Ferm. N 1/50F	12 Tg.	40	36	90	1:10000	1 Std.	40° C.	22	61	17	47	10	27
Kont. N 4/50K	20 Tg.	39	34	87	..	..	20° C.	29	85	18	53	5	15
Ferm. N 4/50F	20 Tg.	39	33	85	1:2500	4 Std.	20° C.	30	91	16	49	3	9

TABELLE 5

Induktionsleistung der Nierenrinde nach Mangelernährung der Spendertiere und nach Ribonucleasebehandlung der Implantate

Serie	Ernährung d. Spender	Alkohol-behandl.	verwertb. Fälle	positive Fälle(n)	Indukt.-rate (%)	Fermentbehandlung			A	%	B	%	C/D	%
						Konz.	Dauer	Temp.						
Kont. N 3/51aK	2 Tg. Hg.	7 Tg.	43	36	84	..	..	40° C.	24	66	14	39	11	31
Ferm. N 3/51aF	2 Tg. Hg.	7 Tg.	55	43	78	1:2500	4 Std.	40° C.	34	79	13	30	7	16
Kont. N 2/51aK	3 Wo. Hg.	5 Tg.	54	49	91	..	..	20° C.	43	88	30	67	6	13
Ferm. N 2/51aF	3 Wo. Hg.	5 Tg.	82	63	66	1:2500	2 Std.	20° C.	31	49	37	62	8	21
Kont. N 5/51K	4 Wo. Hg.	3–5 Std.	55	53	96	..	..	20° C.	48	90	26	49	12	23
Ferm. N 5/51F	4 Wo. Hg.	3–5 Std.	75	51	68	1:2500	3 Std.	20° C.	36	71	35	68	8	16

3. Die Leistungsspezifität der Implantate der Kontrollserien war durch Mangeldiät der Spendertiere verändert (Tab. 5).

(*a*) Das Induktionsmaterial stammt von einem Spendertier, das zwei Tage gehungert hatte. Infolge Alkoholbehandlung (7 Tg.) konnte eine überwiegend archencephale Spezifität festgestellt werden (Serie N 3/51aK).

Nach Fermentbehandlung trat eine Änderung der spezifischen Induktionsleistung und der Induktionsaktivität nicht in Erscheinung (Serie N 3/51aF, Heterogenität nicht signifikant).

(*b*) Das Induktionsmaterial rührte von Spendertieren her, die drei und vier Wochen einer Mangeldiät ausgesetzt waren. Es zeigte eine überwiegend archencephale Leistung (Serie N 2/51aK und N 5/51K).

Durch die Fermentbehandlung wurden sowohl die Leistungsspezifität als auch die Induktionsaktivität verändert (Serie N 2/51aF und N 5/51F). Die Herabsetzung der Induktionsaktivität äußerte sich in erster Linie in einem Sinken der Zahl der archencephalen Komplexe (Heterogenität schwach signifikant Serie N 2/51aF A : nicht A $X^2 = 18{,}27$ $P < 1\%$; Induktionsrate $X^2 = 4{,}34$ $P > 2\%$; Serie N 5/51F A: nicht A $X^2 = 6{,}68$ $P < 1\%$; Induktionsrate $X^2 = 14{,}23$ $P < 1\%$). Im Gegensatz zu den unter 2 besprochenen Versuchen traten Gehirne, Augen und Nasen in einem geringeren Prozentsatz als in den Kontrollserien auf.

Zusammenfassend kann gesagt werden, daß eine Abschwächung der archencephalen Wirksamkeit nur in den Serien, deren Induktionsmaterial von länger hungernden Spendertieren stammte, in Erscheinung trat. In den übrigen Gruppen kam es zu keiner wesentlichen Veränderung der spezifischen Induktionsleistung.

B. *Die Induktionsleistung des Lebergewebes*

1. *Die spezifische Induktionsleistung des kurzfristig mit Alkohol behandelten Lebergewebes unter besonderer Berücksichtigung des Alters der Spendertiere*

Mit der oben angegebenen Methode wurde die Induktionsleistung des Lebergewebes nach kurzfristiger Alkoholbehandlung festgestellt. Als Spendertiere standen Meerschweinchen im Alter von vier Wochen und sechs Monaten zur Verfügung.

Schon die äußere Betrachtung der operierten Keime zeigte, daß die Leistungsspezifität des Lebergewebes von der der Nierenrinde verschieden ist. Sichtbare Induktionsgebilde traten nur in geringer Zahl auf und bestanden aus kleinen Buckeln, oft mit Gehirn und Sinnesorganen. Ohrblasen mit Hörsteinchen kamen nur selten, Schwänze überhaupt nicht zur Beobachtung.

Die histologische Untersuchung (Tab. 6) ergab, daß in erster Linie archencephale Hirnkomplexe mit Augen und Nasen, freie Linsen und Balancer induziert worden waren. Deuterencephale Hirnstrukturen und Ohrblasen konnten nur in einigen Fällen analysiert werden, typisch ausgebildete spinocaudale

Organe fehlten vollkommen. Es wurden aber verhältnismäßig häufig mit Mesenchym gefüllte Epidermisvorwölbungen (Ganglienleistenderivate?) gefunden (Fig. 3).

Tabelle 6

Induktionsleistung des Lebergewebes in Abhängigkeit vom Alter der Spendertiere. (Ernährung der Spendertiere: normal, Alkoholbehandlung der Implantate: 3–5 Std.). Keine C/D-Induktion

Serie	*Alter d. Spender*	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*A*	*B*
L 6/51	4 Wo.	35	24	68	23 96%	7 29%
L 1/51	6 Wo.	44	28	64	28 100%	. . . .
L 3/50	6 Mo.	51	30	59	30 100%	1 3%
L 2/50	6 Mo.	53	27	51	27 100%	. . . .

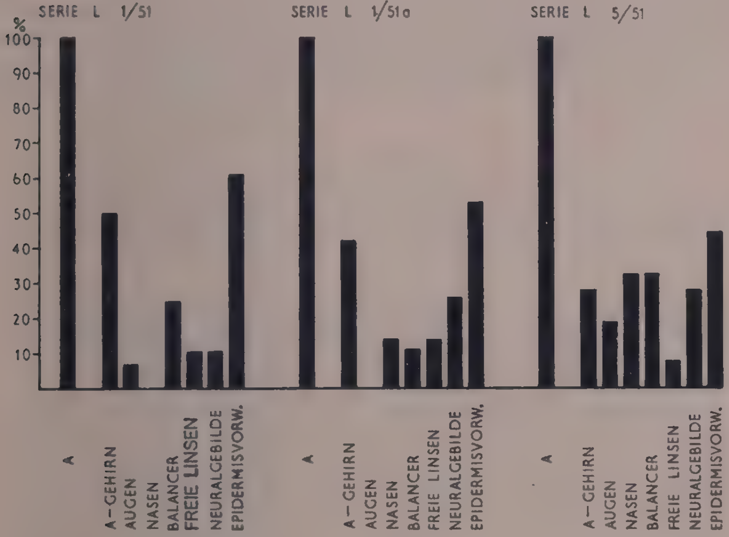

Fig. 3. Induktionsleistung des Lebergewebes vom Meerschweinchen nach kurzer (Serie L 1/51, 3–5 Std.) und nach langfristiger (Serie L 1/51a, 8 Tg.) Alkoholbehandlung der Implantate sowie nach langer Hungerzeit (Serie L 5/51, 4 Wo. Hg.) der Spendertiere.

Der auffälligste Befund war die für alle Serien festgestellte, verhältnismäßig niedrige allgemeine Induktionsrate (68 %–51 %). Ein Vergleich mit den Nierenserien macht diese Tatsache deutlich: Hier lag die Induktionsrate zwischen 96 % und 85 %. Induktionsrate der Leberserien (L 6/51, L 1/51, L 3/50 und L 2/50):

Induktionsrate der Nierenserien (N 7/49, N 6/51 und N 4/50) $X^2 = 40{,}66$ $P \ll 1\,\%$ (Heterogenität signifikant).

Bei einer Analyse der Prozentzahlen der im einzelnen induzierten Organe zeigte es sich, daß nicht nur die quantitative Induktionsleistung bei einem Vergleich mit den Nierenserien, die eine überwiegend archencephale Spezifität aufwiesen, herabgesetzt ist, sondern daß auch die qualitative Zusammensetzung der Induktionsgebilde eine andere ist. Archencephale Gehirnkomplexe, Augen und Nasen treten in einem geringeren Prozentsatz auf.

Eine vom Alter abhängige Veränderung der Leistungsspezifität ließ sich nicht feststellen.

2. *Der Einfluß länger dauernder Alkoholbehandlung auf die Induktionsleistung des Lebergewebes*

Längeres Verbleiben der Nierenimplantate in 70%igem Alkohol hatte bei Prüfung der Leistungsspezifität das Ergebnis erbracht, daß der spinocaudale Komplex verschwindet, gleichzeitig aber der archencephale ansteigt. Um zu entscheiden, ob die Vermehrung der archencephalen Organe direkt von chemisch-physiologischen Veränderungen des Induktionsmaterials abhängig ist oder indirekt durch das Sinken des spinocaudalen Komplexes bedingt wird, erschien es von Interesse, einen Induktor mit überwiegend archencephaler Leistungsspezifität, wie er im Lebergewebe vorliegt, einer länger dauernden Alkoholbehandlung auszusetzen.

TABELLE 7

Induktionsleistung des Lebergewebes in Abhängigkeit von der Dauer der Alkoholbehandlung der Implantate. Keine B- und C/D-Induktionen. (Alter der Spendertiere: 6 Wo.)

Serie	*Alkohol-behandl.*	*Ernährung d. Spender*	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*A*
L 2/51	3–5 Std.	3 Wo. Hg.	63	35	55	35 100%
L 2/51a	3 Tg.	3 Wo. Hg.	51	20	39	20 100%
L 1/51	3–5 Std.	normal	44	28	64	28 100%
L 1/51a	8 Tg.	normal	59	35	59	35 100%

Die in Tab. 7 zusammengestellten Werte zeigen, daß die spezifische Induktionsleistung des Lebergewebes durch eine Denaturierung mit Alkohol nicht beeinflußt wird. Die ausgesprochene archencephale Tendenz ist in vollem Umfang erhalten geblieben (in allen Serien 100%) (Fig. 3). Die Induktionsrate ist in verschiedenem Ausmaß abgesunken. Diese statistisch nicht signifikante (Induktionsrate $X^2 = 0{,}18$ $P > 50\,\%$) Schwächung der Induktionsaktivität kann vielleicht darauf beruhen, daß der für die archencephale Leistung verantwortliche Faktor in geringem Maße durch länger dauernde Alkoholbehandlung ausgewaschen werden kann, was in den Experimenten mit Nierenrinde nicht in Erscheinung trat, da hier immer im Gegensatz zum Lebergewebe eine ausgesprochen starke Induktionswirkung vorhanden war.

3. *Der Einfluß von Hunger bzw. Eiweißmangeldiät der Spendertiere auf die Induktionsleistung des Lebergewebes*

Die Experimente, die Aufschluß darüber bringen sollten, welchen Einfluß Mangelernährung der Spendertiere auf die Induktionsleistung der Nierenrinde hat, hatten zu dem Resultat geführt, daß der archencephale Komplex vermehrt auftritt, während der spinocaudale fast vollständig zum Verschwinden gebracht wird.

Tabelle 8

Induktionsleistung des Lebergewebes in Abhängigkeit vom Ernährungszustand der Spendertiere (Alkoholbehandlung der Implantate: 3–5 Std.). Keine B- und C/D-Induktionen

Serie	*Ernährung d. Spender*	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*A*
L 1/51	normal	44	28	64	28 100%
L 3/51	2 Tg. Hg.	58	30	52	30 100%
L 2/51	3 Wo. Hg.	63	35	55	35 100%
L 5/51	4 Wo. Hg.	55	36	65	36 100%

Die Ergebnisse der entsprechenden Versuche mit Lebergewebe sind in Tab. 8 zusammengestellt. Eine Änderung der Leistungsspezifität in Abhängigkeit vom Hunger bzw. Mangelernährung der Spendertiere ist nicht zu erkennen. Die archencephale Leistung ist in allen Serien in gleichem Ausmaß (100%) vorhanden (Fig. 3). Die im einzelnen induzierten Organe schwanken in ihrer Zahl, ohne daß eine Regelmäßigkeit festzustellen ist. Nur die Gehirne zeigen eine geringe zahlenmäßige Abnahme.

4. *Die Veränderung der Induktionsleistung des Lebergewebes nach Behandlung mit Ribonuclease*

Ribonucleasebehandlung der Nierenrinde-Implantate hatte nur nach langdauernder Mangelernährung der Spendertiere zu einem Abfall der archencephalen Induktionsleistung geführt. Im folgenden sollte festgestellt werden, welche Veränderung der Induktionsleistung nach einer Fermentbehandlung des Lebergewebes, das, wie vorher gezeigt wurde, ein archencephal wirkender Induktor mit verhältnismäßig geringer Induktionsaktivität ist, auftritt.

Fermentkonzentration. Einwirkungszeit und Temperatur sind in Tab. 9 für die einzelnen Versuchsgruppen aufgeführt. Als auffälligster Befund konnte bei einem Vergleich von Kontroll- und Fermentserien ein Sinken der Induktionsrate festgestellt werden. in der Gruppe L 3/50K und F von 59 % auf 24 % (Heterogenität signifikant $\chi^2 = 12.60$ P $<$ 1 %). in der Gruppe L 2/50K und F von 51 % auf 30 % (Heterogenität schwach signifikant $\chi^2 = 4.54$ P $>$ 2 %) und in der Gruppe L 2/51K und F von 39 % auf 27 % (Heterogenität nicht signifikant $\chi^2 = 1.92$ P $>$ 10 %). Mit der Schwächung der Induktionsaktivität ist eine qualitative Veränderung des A-Komplexes verbunden. In erster Linie treten die archencephalen Hirnteile sowie die Sinnesorgane Augen und Nasen in geringerer Zahl auf bzw. sind ganz verschwunden (Fig. 4). Eine vollkommene Auslöschung der neuralen Induktionsfähigkeit (Brachet. 1942) konnte nicht erzielt werden.

TABELLE 9

Induktionsleistung des Lebergewebes nach Ribonucleasebehandlung der Implantate. Keine B- und C/D-Induktionen

Serie	*Ernährung d. Spender*	*Alkohol-behandl.*	*verwerth. Fälle*	*positive Fälle (n)*	*Indukt.-rate (%)*	***Fermentbehandlung*** *Konz.*	*Dauer*	*Temp.*	*A*	*%*
Kont. L 3/50K	**normal**	**3–5 Std.**	**51**	**30**	**59**	..	..	**20° C.**	**30**	**100**
Ferm. L 3/50F	normal	3–5 Std.	50	12	24	1:2500	2 Std.	20° C.	12	100
Kont. L 2/50K	normal	3–5 Std.	53	27	51	..	..	20° C.	27	100
Ferm. L 2/50F	normal	3–5 Std.	44	13	30	1:5000	1 Std.	20° C.	13	100
Kont. L 2/51K	3 Wo. Hg.	3 Tg.	51	20	39	..	..	20° C.	20	100
Ferm. L 2/51F	3 Wo. Hg.	3 Tg.	73	20	27	1:2500	4 Std.	20° C.	20	100

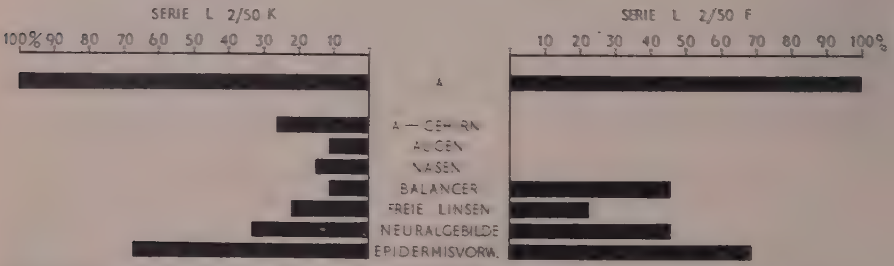

FIG. 4. Induktionsleistung des Lebergewebes vom Meerschweinchen nach Ribonucleasebehandlung der Implantate (Serie L 2/50K = Kontrollserie. Serie L 2/50F = Fermentserie).

II. Die Beeinflußbarkeit der Leistungsspezifität von Parotis- und Herzmuskelimplantaten

von ANNA GISELA JOHNEN

A. *Die Induktionsleistung der Parotis vom Meerschweinchen nach unterschiedlich langer Alkoholbehandlung der Implantate*

Die Implantate der folgenden Versuchsserien, die von fünf normal ernährten Spendertieren stammen, wurden unterschiedlich lange in 70%igem Äthylalkohol fixiert. Aus diesen Versuchen wurden 308 verwertbare Fälle gewonnen, von denen sich 277 als positiv erwiesen haben (Tab. 10).

1. *Alkoholeinwirkung während 3–5 Stunden*

Die Implantate, die nur kurz in Alkohol abgetötet worden sind, besitzen eine sehr hohe Induktionsaktivität, die allgemeine Induktionsrate beträgt 97%.

TABELLE 10

Induktionsleistung der Parotis nach verschieden langer Alkoholfixierung der Implantate (Ernährung der Spendertiere: normal)

Serie	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*Alkohol-behandl.*	*A*	*B*	*C/D*
I	66	64	97	3–5 Std.	14	57	62
					21%	89%	97%
II	96	86	89	1 Tg.	12	80	62
					14%	93%	72%
III	62	51	82	3 Tg.	19	44	9
					37%	86%	17%
IV	39	33	84	5 Tg.	31	12	3
					94%	36%	9%
V	45	43	95	14 Tg.	40	20	..
					93%	46%	..

Der Induktionserfolg ist ein überwiegend deuterencephal-spinocaudaler, archencephale Induktionsgebilde sind nur in einem verhältnismäßig geringen Prozentsatz vorhanden (Tab. 10, Serie I).

Spinocaudale Induktionsgebilde treten in 97% der Fälle auf. Meistens handelt es sich um vollständige Schwänze mit Chorda, Neuralrohr, Muskulatur und Flossensaum (70%).

Die deuterencephalen Gebilde, die fast in gleich hohem Prozentsatz (89%) auftreten, sind sehr häufig mit spinocaudalen Organen kombiniert. Sie bilden so mit diesen zusammen komplette Hinterkopf-Rumpfschwanzgebilde.

Den geringsten Anteil an dem Induktionsergebnis haben die archencephalen Gebilde mit nur 21%. Es erscheinen immer archencephale Organkomplexe, wie Vorder- und Zwischenhirn mit Augen, Nasen und Balancer. Ferner sind diese in jedem Fall mit deuterencephalen bzw. deuterencephal-spinocaudalen Induktionskomplexen kombiniert, so daß neben den vorhin beschriebenen Hinterkopf-Rumpfschwanzgebilden in einigen Fällen nahezu vollständige sekundäre Embryonalanlagen entstanden sind.

2. *Alkoholeinwirkung während 1 und 3 Tagen*

Nach einer 1- und 3-tägigen Alkoholfixierung der Implantate zeigt sich eine weitgehende Änderung der induktiven Fähigkeit (Tab. 10, Serie II und III).

Die Leistungsfähigkeit der Parotis in bezug auf die spinocaudale Leistung hat beträchtlich nachgelassen. Die spinocaudale Induktionsrate ist nach 3-tägiger Alkoholbehandlung auf 17% gesunken, vollständige Schwänze sind nur noch in 10% der Fälle vorhanden.

In dem gleichen Maße, wie die spinocaudale Induktionswirkung abnimmt, steigt die Tendenz zur archencephalen Leistung der Implantate an. Nach 3-tägiger Alkoholfixierung beträgt die archencephale Induktionsrate 37% und übertrifft damit die der spinocaudalen, während es in den vorhergehenden Serien umgekehrt war.

Die deuterencephale Induktionsrate wird nicht beeinflußt.

3. *Alkoholeinwirkung während 5 und 14 Tagen*

Die schon vorhin angedeutete Veränderung der Induktionsfähigkeit der Implantate tritt nach längerer Alkoholfixierung noch deutlicher in Erscheinung (Tab. 10, Serie IV und V).

Die spinocaudale Induktionstendenz erfährt eine weitere Abschwächung. Nach 14-tägiger Alkoholfixierung treten keine spinocaudalen Organe mehr auf.

Die archencephale Induktionsleistung der Implantate nimmt weiter zu und erreicht nach 14-tägiger Alkoholbehandlung 93%.

Nach 5-tägiger Alkoholbehandlung macht sich eine Veränderung in der deuterencephalen Induktionsleistung der Implantate bemerkbar. Der Prozentsatz der induzierten deuterencephalen Gebilde sinkt auf 46% nach 14-tägiger Alkoholfixierung herab. Dieser Abfall beruht hauptsächlich auf einer Verminderung der deuterencephalen Hirnteile, die nur noch in 14% vorhanden sind.

4. *Zusammenfassende Betrachtung über die Bedeutung verschieden langer Alkoholfixierung für das Induktionsvermögen der Parotis*

In den Untersuchungen wurde festgestellt, daß die alkoholfixierte Parotis befähigt ist, sämtliche Organkomplexe im Ektoderm der frühen Gastrula zu induzieren. Es hat sich aber ergeben, daß eine weitgehende Abhängigkeit des Induktionsvermögens von der Dauer der Alkoholbehandlung der Implantate besteht.

Die Veränderungen in der Induktionsleistung der Parotis vollziehen sich bei gleicher hoher allgemeiner Induktionsrate. Die Induktionsaktivität der Parotis wird, wie ein Vergleich der Induktionsrate von Serie I (97%) und Serie V (95%)

zeigt, durch langdauernde Alkoholeinwirkung nicht beeinflußt (Homogenität signifikant P > 80%).

In Fig. 5 sind die Prozentzahlen der induzierten Organkomplexe nach kurzer (3–5 Std.) und 14-tägiger Alkoholfixierung gegenübergestellt, um das Gegensätzliche in der Induktionsleistung aufzuzeigen. Man sieht daraus, daß die Parotis nach kurzer Alkoholbehandlung überwiegend deuterencephal-spinocaudal induziert und daß eine 14-tägige Alkoholfixierung die Fähigkeit zur Induktion spinocaudaler Organkomplexe vernichtet. Gleichzeitig steigt die Tendenz zur archencephalen Induktion an. Aus der Besprechung der einzelnen Serien geht hervor, daß der Anstieg des archencephalen Induktionsvermögens in der Hauptsache erst dann erfolgt, wenn die Tendenz zur spinocaudalen Induktion nach 3-tägiger Alkoholeinwirkung fast vernichtet ist. Dies scheint zu bedeuten, daß die archencephale Induktionswirkung erst nach Abschwächung bzw. Vernichtung der spinocaudalen voll zur Wirkung kommen kann.

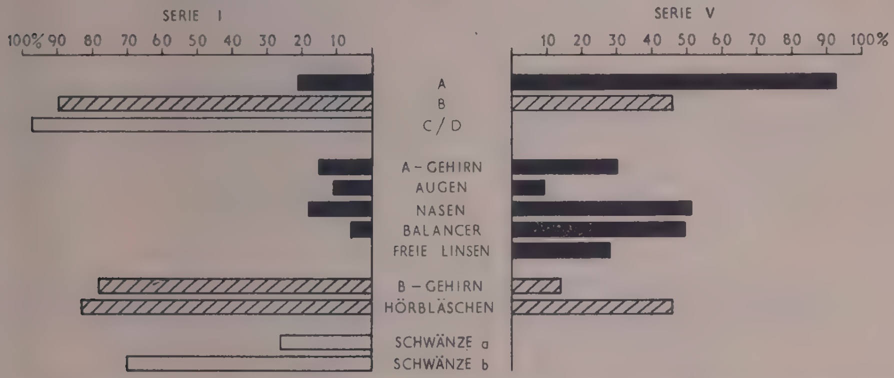

FIG. 5. Induktionsleistung der Parotis vom Meerschweinchen nach kurzer (Serie I, 3–5 Std.) und nach langfristiger (Serie V, 14 Tg.) Alkoholbehandlung der Implantate. Ernährung der Spendertiere: normal. (Schwänze a = unvollständige Schwanzgebilde, Schwänze b = vollständige Schwänze mit Chorda, Neuralrohr, Muskulatur und Flossensaum.)

Aus Fig. 5 geht ferner hervor, daß die einzelnen archencephalen Organe eine unterschiedliche Zunahme ihrer prozentualen Häufigkeit aufweisen. Die Augen zeigen keine Vermehrung, während archencephale Hirnteile und Nasen einen prozentualen Anstieg auf das Doppelte bzw. Dreifache erfahren. Freie Linsen und Balancer zählen nach 14-tägiger Alkoholbehandlung neben Nasen und Hirnteilen zu den häufigen Induktionsgebilden, während sie nach kurzer Alkoholeinwirkung kaum in Erscheinung treten.

Die deuterencephale Induktionsfähigkeit wird durch langdauernde Alkoholbehandlung ebenfalls beeinflußt. Nach 14-tägiger Alkoholeinwirkung ist die Zahl der induzierten deuterencephalen Gebilde auf fast die Hälfte vermindert.

Als Endergebnis dieser Versuchsserien kann festgestellt werden, daß durch unterschiedlich lange Alkoholbehandlung der Parotis die Induktionsleistung dieses heterogenen Induktors geändert wird. Die nach kurzer Alkoholeinwirkung in Erscheinung tretende überwiegend deuterencephal-spinocaudale Induktionswirkung wird bei länger dauernder Alkoholfixierung in eine archencephal-deuterencephale Induktionstendenz verwandelt (Heterogenität signifikant A : nicht A $X^2 = 143{,}95$ $P \ll 1\%$, B : nicht B $X^2 = 73{,}33$ $P \ll 1\%$, C/D: nicht C/D $X^2 = 117{,}73$ $P \ll 1\%$).

B. *Die Induktionsleistung der Herzmuskulatur vom Meerschweinchen nach unterschiedlich langer Alkoholbehandlung der Implantate*

In diesen Versuchsreihen wurden 602 verwertbare Fälle gewonnen, von denen 438 Keime einen positiven Induktionseffekt aufweisen. Die Implantate stammen von sechs normal ernährten Meerschweinchen im Alter von drei Wochen bis sechs Monaten und wurden unterschiedlich lange mit 70%igem Äthylalkohol fixiert (Tab. 11).

TABELLE 11

Induktionsleistung der Herzmuskulatur nach verschieden langer Alkoholfixierung der Implantate (Ernährung der Spendertiere: normal)

Serie	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*Alkohol-behandl.*	*A*	*B*	*C/D*
A	40	28	70	12 Std.	22	14	3
					78%	50%	10%
B	80	61	76	1 Tg.	55	33	6
					90%	54%	9%
C	46	31	67	6 Tg.	27	10	5
					87%	32%	15%
D	157	126	80	7 Tg.	109	69	12
					86%	54%	19%
E	177	133	75	10 Tg.	123	53	3
					92%	45%	4%
F	102	59	58	13 Tg.	55	15	3
					93%	25%	4%

Die Induktionsaktivität der Herzmuskulatur ist bedeutend geringer als die der Parotis. Die allgemeine Induktionsrate schwankt bei den einzelnen Serien beträchtlich. Langdauernde Alkoholbehandlung scheint jedoch keinen Einfluß auf die Induktionsaktivität zu haben, wie ein Vergleich der Induktionsrate von Serie A (70%) und Serie F (58%) zeigt (Homogenität signifikant $P > 10\%$).

In Fig. 6 sind die Ergebnisse nach kurzfristiger (12 Std.) und langfristiger (13 Tg.) Alkoholbehandlung gegenübergestellt. Man sieht, daß die Herzmuskulatur nach kurzer Alkoholfixierung zu überwiegend archencephalen Induktionsleistungen befähigt ist. Nach länger dauernder Alkoholbehandlung bleibt die archencephale Induktionsleistung in vollem Umfang erhalten (Homogenität signifikant A : nicht A $X^2 = 6{,}99$ $P > 20\%$). Den größten Anteil an der Zusammensetzung des archencephalen Induktionskomplexes haben nach kurzer Alkoholfixierung die Nasen mit 46%, aber auch archencephale Hirnteile, Augen und freie Linsen sind in verhältnismäßig großem Prozentsatz vorhanden, Balancer dagegen wurden nicht realisiert. Diese Zusammensetzung des archencephalen Komplexes bleibt auch nach langer Alkoholeinwirkung fast unverändert. Der Prozentsatz der archencephalen Hirnteile und freien Linsen bleibt konstant, vermindert ist die Häufigkeit der induzierten Nasen und Augen. Nach längerer Alkoholbehandlung treten auch Balancer auf.

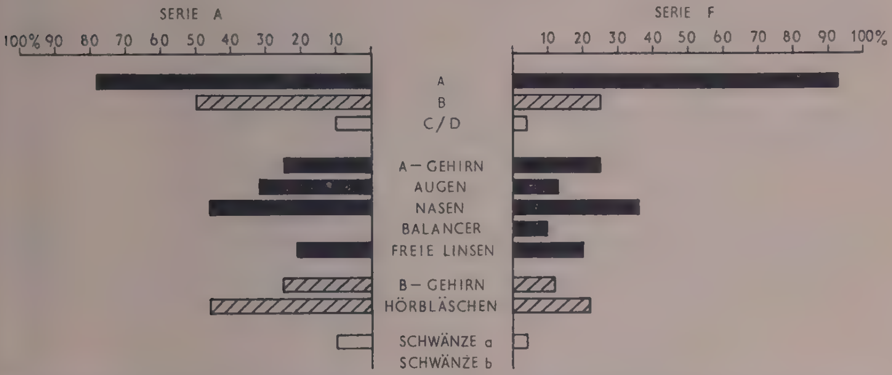

FIG. 6. Induktionsleistung der Herzmuskulatur vom Meerschweinchen nach kurzer (Serie A, 12 Std.) und nach langfristiger (Serie F, 13 Tg.) Alkoholbehandlung der Implantate. Ernährung der Spendertiere: normal.

Die spinocaudale Tendenz ist bei diesem Induktor sowohl nach kurzer als auch nach längerer Alkoholbehandlung nur sehr schwach vorhanden. Sie tritt kaum in Erscheinung, ein vollständiger Schwanz mit Chorda, Neuralrohr, Muskulatur und Flossensaum konnte nicht identifiziert werden. Wie aus Fig. 6 hervorgeht, wird die spinocaudale Induktionsleistung nach langer Alkoholeinwirkung noch etwas vermindert.

Die deuterencephale Wirkung des Induktors wird durch die Dauer der Alkoholfixierung am meisten beeinflußt. Während nach kurzer Alkoholbehandlung die deuterencephale Induktionsrate 50% beträgt, sinkt sie nach 13-tägiger Alkoholeinwirkung auf 25% herab (Heterogenität signifikant B : nicht B $X^2 = 19{,}73$ $P < 1\%$).

Abschließend läßt sich sagen: Herzmuskulatur ist ein überwiegend archencephal wirkender Induktor mit einer verhältnismäßig geringen deuterencephalspinocaudalen Tendenz. Dieses Wirkungsbild wird durch langdauernde Alkoholbehandlung der Implantate nicht wesentlich beeinflußt.

Es sei noch erwähnt, daß das Alter der Spendertiere in den hier beschriebenen Versuchsserien keinen feststellbaren Einfluß auf die Induktionsleistung der Parotis und Herzmuskulatur ausübt.

C. *Die Induktionsleistung der Parotis vom Meerschweinchen nach unterschiedlich langer Hungerzeit der Spendertiere*

Die Implantate der folgenden Versuchsserien stammen von Meerschweinchen, die unterschiedlich lange Eiweißmangeldiät erhielten. Sämtliche hier verwendeten Implantate wurden nur kurz (3–5 Std.) in 70%igem Äthylalkohol fixiert, so daß eine Veränderung zu Gunsten der archencephalen Induktionsleistung, wie sie nach längerer Alkoholbehandlung festgestellt wurde, ausgeschlossen ist. Für diese fünf Versuchsserien stehen 299 verwertbare Fälle zur Verfügung, von denen sich 283 als positiv erwiesen haben (Tab. 12).

TABELLE 12

Induktionsleistung der Parotis bei normaler Ernährung und nach verschieden langer Hungerzeit der Spendertiere (Alkoholbehandlung der Implantate: 3–5 Std.)

Serie	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*Ernährung d. Spenders*	*A*	*B*	*C/D*
VI (=I)	66	64	97	normal	14 21%	57 89%	62 97%
VII	73	68	93	1 Tg. Hg.	19 28%	63 93%	55 81%
VIII	64	61	95	2 Tg. Hg.	37 57%	55 90%	23 37%
IX	49	47	96	3 Wo. Hg.	32 68%	44 93%	19 40%
X	47	43	91	4 Wo. Hg.	33 76%	38 88%	7 16%

1. *Die Induktionsleistung bei normaler Ernährung*

Diese, mit der Serie I der Alkoholbehandlung identische Serie VI wurde bereits dort (S. 15) ausführlich besprochen. Die Ergebnisse waren kurz folgende: Die allgemeine Induktionsrate ist sehr hoch und zeigt dadurch eine gute Induktionsaktivität der Implantate an. Das Parotis-Gewebe erweist sich nach kurzer Alkoholbehandlung als ein Induktor mit einer ausgesprochen deuterencephal-spinocaudalen Wirkung. Archencephale Induktionsgebilde treten nur in geringem Prozentsatz auf (Tab. 12, Serie VI).

2. *Kurze Hungerperiode (1 und 2 Tage)*

Nach kurzer Hungerzeit ist eine deutliche Veränderung der Induktionsfähigkeit der Parotis bei gleichbleibender hoher Induktionsaktivität festzustellen (Tab. 12, Serie VII und VIII).

Die Induktionsrate des C/D-Komplexes ist von 97% in Serie VI auf 37% in Serie VIII gesunken. Vollständige Schwänze sind nur noch in 11% der Fälle, gegenüber 70% in Serie VI, vorhanden.

Dagegen ist die deuterencephale Induktionsleistung der Implantate erhalten geblieben.

Die archencephale Induktionsrate ist gegenüber der Serie VI ganz bedeutend von 21% auf 57% in Serie VIII angestiegen. Die archencephale Induktionsleistung der Implantate ist somit mehr als verdoppelt worden und übertrifft die spinocaudale ganz erheblich. Nach 2-tägigem Hunger treten alle archencephalen Organe in vermehrter Anzahl auf. Diese Erhöhung ist besonders deutlich bei den Hirnteilen, Nasen und Balancern.

3. *Lange Hungerperiode (3 und 4 Wochen)*

Die in den vorhergehenden Versuchsserien festgestellten Veränderungen in der Induktionsleistung werden noch deutlicher, wenn die Parotis-Spender längere Zeit gehungert haben (Tab. 12, Serie IX und X).

Serie X zeigt bei gleicher allgemeiner Induktionsrate (91%) eine weitere Abschwächung der spinocaudalen Wirkung der Implantate. Spinocaudale Induktionsgebilde treten nur noch in 16% der Fälle auf.

Der deuterencephale Induktionskomplex behält weiter seine hohe Induktionsrate (88%) in Serie X bei.

Die archencephale Wirkung der Parotis ist weiter gesteigert worden. Nach 4-wöchentlicher Hungerperiode beträgt die Induktionsrate 76%.

4. *Zusammenfassende Betrachtung über die Bedeutung des Ernährungszustandes des Spendertieres für das Induktionsvermögen der Parotis*

Durch Veränderung des physiologisch-chemischen Zustandes von Parotis-Gewebe mittels Eiweißmangeldiät der Spendertiere konnte eine Beeinflussung der Induktionsleistung der Implantate erzielt werden.

Die Veränderung in der Induktionsleistung der Parotis erfolgt bei gleicher hoher allgemeiner Induktionsrate. Ein Vergleich der Induktionsraten bei normaler Ernährung (Serie VI, 97%) und nach 4-wöchentlicher Hungerzeit (Serie X, 91%) zeigt, daß die Induktionsaktivität der Implantate durch Mangeldiät der Spendertiere nicht beeinflußt wird (Homogenität signifikant $P > 30\%$).

In Fig. 7 sind die Induktionsergebnisse bei normalem Ernährungszustand des Spendertieres und nach 4-wöchentlicher Hungerperiode zum Vergleich nebeneinander gestellt. Man sieht, daß die bei normaler Ernährung vorhandene hohe spinocaudale Induktionstendenz der Implantate nach langer Hungerzeit ganz beträchtlich vermindert ist. Die spinocaudale Induktionsrate ist von 97% in Serie VI auf 16% in Serie X gesunken. Gleichzeitig steigt die Tendenz zur archencephalen Induktion an. Die Besprechung der einzelnen Serien hat gezeigt, daß das Ansteigen der archencephalen Induktionsfähigkeit hauptsächlich erst nach Abschwächung der spinocaudalen Tendenz der Implantate erfolgt.

Fig. 7 zeigt weiter, daß alle archencephalen Organe nach langer Hungerzeit vermehrt auftreten. Die Hirnteile zeigen die größte Steigerung, sie sind von 15% in Serie VI auf 67% in Serie X angestiegen. Augen und Nasen haben ihr Vorkommen prozentual verdoppelt. Nach langer Hungerzeit treten auch 21% Balancer auf, während freie Linsen in beiden Serien kaum in Erscheinung treten.

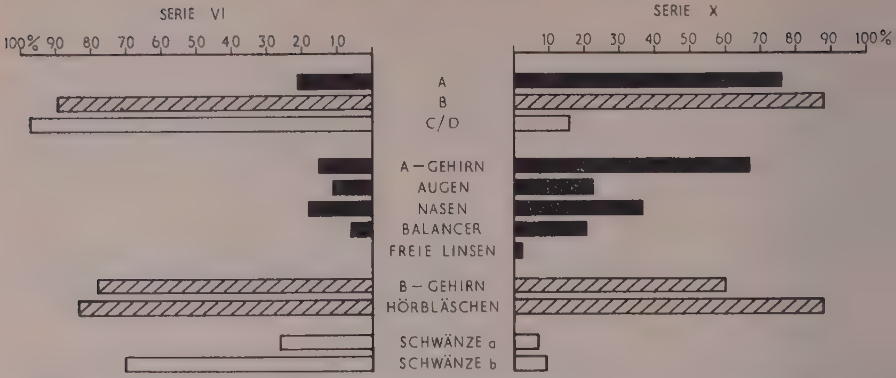

FIG. 7. Induktionsleistung der Parotis vom Meerschweinchen bei normaler Ernährung (Serie VI) und nach langer Hungerzeit (Serie X, 4 Wo. Hg.) der Spendertiere. Alkoholbehandlung der Implantate: 3–5 Std. (Schwänze a = unvollständige Schwanzgebilde, Schwänze b = vollständige Schwänze.)

Die deuterencephale Induktionswirkung bleibt in ihrer Gesamtheit unbeeinflußt (Homogenität signifikant B : nicht B $X^2 = 1{,}26$ $P > 80\,\%$). Deuterencephale Hirnteile nehmen nach langer Hungerperiode etwas ab (von 78 % in Serie VI auf 60 % in Serie X), Hörbläschen treten dagegen in leicht vermehrter Anzahl auf (83 % in Serie VI, 88 % in Serie X).

Als Endergebnis dieser Versuchsserien kann festgestellt werden, daß die Leistungsspezifität der Parotis durch Eiweißmangeldiät der Spendertiere weitgehend beeinflußt wird. Die Veränderungen äußern sich in ähnlicher Weise wie nach langdauernder Alkoholbehandlung der Implantate: Aus einem überwiegend deuterencephal-spinocaudal wirkenden Induktor wird nach langer Hungerzeit ein archencephal-deuterencephal induzierendes Gewebe (Heterogenität signifikant A : nicht A $X^2 = 52{,}42$ $P \ll 1\,\%$, C/D : nicht C/D $X^2 = 101{,}73$ $P \ll 1\,\%$.

D. *Die Induktionsleistung der Parotis und Herzmuskulatur vom Meerschweinchen nach Behandlung mit dem Ferment Ribonuclease*

Ausgehend von der Vermutung Brachet's (1942), daß die Ribonucleinsäure von besonderer Bedeutung für den neuralen Induktionsvorgang ist, wurde in einigen Versuchsserien geprüft, ob eine Behandlung der Implantate vor dem Einstecken mit dem Ferment Ribonuclease eine Wirkung auf die Induktionsleistungen der Parotis und Herzmuskulatur ausübt.

In den hier vorliegenden Experimenten wurde jeder Fermentserie eine Kontrollserie gegenübergestellt, die unter sonst gleichen experimentellen Bedingungen an Stelle der Fermentbehandlung mit sterilisiertem Aqua bidest. behandelt wurde. Für diese Versuchsserien kommen einschließlich der Kontrollen 926 verwertbare Fälle in Betracht, von denen 659 Keime einen positiven Induktionseffekt aufweisen.

Die Experimente lassen sich auf Grund der leistungsspezifischen Wirkung des Ausgangsmaterials in vier Gruppen einteilen.

1. Fermentbehandlung von überwiegend deuterencephal-spinocaudal induzierender Parotis nach kurzfristiger Alkoholbehandlung bewirkt eine geringe Erhöhung der archencephalen Tendenz (Heterogenität schwach signifikant A:nicht A $X^2=5{,}39$ $P>2\%$), während die deuterencephale und spinocaudale Leistung der Implantate nicht beeinflußt wird (Heterogenität nicht signifikant). Die archencephale Induktionsrate wird von 21% in der Kontrollserie auf 41% in der Fermentserie erhöht. Alle archencephalen Organe treten nach Fermentbehandlung in leicht vermehrter Anzahl auf, besonders die Hirnteile (Tab. 13).

Die Induktionsaktivität der Implantate bleibt unverändert (Homogenität signifikant $P>90\%$).

Tabelle 13

Induktionsleistung der kurzfristig (3–5 Std.) mit Alkohol fixierten Parotis nach Ribonucleasebehandlung der Implantate

Serie	verwertb. Fälle	positive Fälle(n)	Indukt.-rate (%)	Fermentbehandlung			A	B	C/D
				Konz.	Dauer	Temp.			
Ferm. 5	59	58	98	1:2500	5 Std.	20° C.	24 (41%)	56 (96%)	56 (96%)
Kont. 5a (=I)	66	64	97	..	..	..	14 (21%)	57 (89%)	62 (97%)

Tabelle 14

Induktionsleistung der langfristig (8–10 Tg.) mit Alkohol fixierten Parotis nach Ribonucleasebehandlung der Implantate. Keine C/D-Induktion

Serie	verwertb. Fälle	positive Fälle(n)	Indukt.-rate (%)	Fermentbehandlung			A	B
				Konz.	Dauer	Temp.		
Ferm. 1	64	31	48	1:10000	1 Std.	40° C.	25 (86%)	10 (32%)
Kont. 1a	54	28	52	..	..	..	27 (96%)	8 (28%)
Ferm. 2	67	50	74	1:5000	2 Std.	40° C.	42 (84%)	27 (54%)
Kont. 2a	47	36	76	..	..	..	27 (75%)	17 (47%)
Ferm. 3	65	34	52	1:2500	2 Std.	40° C.	23 (67%)	13 (38%)
Kont. 3a	41	22	53	..	..	..	20 (91%)	4 (18%)
Ferm. 4	61	61	100	1:2500	2 Std.	40° C.	59 (96%)	22 (36%)
Kont. 4a	13	11	89	..	..	..	9 (90%)	3 (30%)

2. Überwiegend archencephal induzierendes Ausgangsgewebe der Parotis nach langfristiger Alkoholbehandlung wird durch die Fermenteinwirkung in seiner spezifischen Leistung nicht beeinflußt (Tab. 14, Heterogenität nicht signifikant). Die archencephale Induktionsrate liegt in den Kontroll- und Fermentserien fast gleich hoch, nur in Serie 3, 3a ist eine sehr geringe Herabsetzung in der archencephalen Induktionsleistung festzustellen.

Unterschiede in der deuterencephalen Induktionsleistung zwischen den Kontroll- und Fermentserien treten nicht auf.

Die allgemeine Induktionsrate bleibt innerhalb der einzelnen Kontroll- und Fermentserien gleich.

3. Ribonucleasebehandlung von Hungergewebe (Parotis: 2 Tage Hunger) mit verhältnismäßig hoher archencephaler Induktionsleistung bleibt ohne Einfluß auf die archencephale Tendenz der Implantate (Tab. 15, Heterogenität nicht signifikant). In der Fermentserie treten aber weniger archencephale Hirnteile auf, die Anzahl der freien Linsen ist dagegen erhöht.

Die deuterencephale Wirkung der Implantate sowie die Induktionsaktivität werden nicht beeinflußt.

TABELLE 15

Induktionsleistung der Parotis von einem Hungertier (2 Tg. Hg.) nach Ribonucleasebehandlung der Implantate. Keine C/D-Induktion

Serie	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*Fermentbehandlung*			*A*	*B*
				Konz.	*Dauer*	*Temp.*		
Ferm. 6	55	31	56	1:2500	6 Std.	40° C.	21 (67%)	12 (38%)
Kont. 6a	36	22	61	..	..	..	14 (63%)	8 (36%)

TABELLE 16

Induktionsleistung der mit Alkohol fixierten (1–11 Tg.) Herzmuskulatur nach Ribonucleasebehandlung der Implantate. Keine C/D-Induktion

Serie	*verwertb. Fälle*	*positive Fälle(n)*	*Indukt.-rate (%)*	*Fermentbehandlung*			*A*	*B*
				Konz.	*Dauer*	*Temp.*		
Ferm. 7	50	36	72	1:10000	2 Std.	40° C.	27 (75%)	17 (47%)
Kont. 7a	32	18	56	..	..	..	15 (83%)	9 (50%)
Ferm. 8	78	65	83	1:5000	2 Std.	40° C.	46 (70%)	36 (55%)
Kont. 8a	37	31	84	..	..	..	23 (74%)	18 (58%)
Ferm. 9	57	27	47	1:2500	2 Std.	40° C.	23 (85%)	6 (22%)
Kont. 9a	44	35	79	..	..	..	32 (91%)	13 (37%)

4. Fermentbehandlung der überwiegend archencephal induzierenden Herzmuskulatur ruft keine Veränderung in der spezifischen Wirkung der Implantate hervor (Tab. 16). Weder die hohe archencephale Induktionsrate noch die deuterencephale Induktionsleistung werden beeinflußt (Heterogenität nicht signifikant). Die Induktionsaktivität der Herzmuskulatur erfährt in Serie 9, 9a nach Ribonucleaseeinwirkung eine Abschwächung (Heterogenität signifikant $P < 1\%$), während sie in den übrigen Serien unbeeinflußt ist (Heterogenität nicht signifikant).

Eine Zusammenfassung der Ergebnisse aller Fermentserien ergibt folgendes: Eine Beeinflussung der Induktionsaktivität der Implantate konnte, so verschieden die spezifischen Leistungen des Ausgangsgewebes auch waren, nur in einer Serie (9, 9a) beobachtet werden, in allen übrigen Serien bleibt sie unverändert.

Die Veränderungen in der Induktionsleistung der Implantate, die durch Ribonucleasebehandlung auftreten, sind gering. Es besteht die Tendenz, daß ein gering archencephal induzierendes Ausgangsgewebe in seiner archencephalen Leistung nach Ribonucleasebehandlung gesteigert wird. Implantate mit einer verhältnismäßig hohen archencephalen Wirkung können, wie Serie 3, 3a zeigt, eine geringe Herabsetzung ihrer archencephalen Leistung erfahren. Vielleicht deutet sich diese Erscheinung in der Veränderung der Zusammensetzung des archencephalen Organkomplexes (Abnahme der Zahl der induzierten archencephalen Hirnteile), wie sie nach Ribonucleasebehandlung von überwiegend archencephal induzierendem Hungergewebe auftritt, und in der Erniedrigung der Induktionsaktivität der Herzmuskulatur in Serie 9, 9a schon an.

Sowohl die deuterencephale als auch die spinocaudale Induktionsfähigkeit der Implantate werden durch die Ribonucleaseeinwirkung nicht beeinflußt.

DISKUSSION

Chuang (1939, 1940) hat gezeigt, daß es möglich ist, die Leistungsspezifität heterogener Induktoren durch Kochen zu ändern. Rotmann's Versuche (1942, 1950) hatten darüber hinaus zu dem Ergebnis geführt, daß die spezifische Wirkung der Implantate verschieden sein kann je nach dem Alter der Spendertiere. Er fand bei den von ihm verwandten Induktoren, Thymus und Mediastinaldrüse, eine deutliche Altersabhängigkeit. Unsere eigenen Versuche führten im Gegensatz dazu zu dem Ergebnis, daß die Nierenrinde- und Leberimplantate ihre Leistungsspezifität behielten, auch wenn das Alter der Spendertiere verschieden war. Auf welcher Ursache diese Diskrepanz beruht, ist vorläufig schwer zu erklären, da einmal von uns andere Organe benutzt wurden und außerdem über die die Induktionen bedingenden Faktoren und ihre Veränderung im Laufe der Entwicklung der Organismen noch keine eindeutigen Aussagen gemacht werden können.

Dagegen war es möglich, in Übereinstimmung mit den Feststellungen Chuang's (1939, 1940) und Toivonen's (1940, 1951) zu zeigen, daß durch physiologisch-chemische Beeinflussung des Induktionsmaterials dessen Leistungsspezifität verändert werden kann. Alkoholbehandlung der Implantate oder Mangeldiät der Spendertiere hatte bei Verwendung von Nierenrinde und Parotis als Induktoren zur Folge, daß die ursprünglich vorhandene deuterencephal-spinocaudale Spezifität in eine archencephal-deuterencephale umgewandelt wurde. Im Gegensatz dazu war die archencephale Leistung des Lebergewebes und der Herzmuskulatur durch die genannten Manipulationen nicht zu beeinflussen. Unsere

Befunde stimmen im wesentlichen mit den Ergebnissen von Vahs (Engländer, Johnen & Vahs, 1953; Vahs, 1957) überein, der mit Mäuseniere und Triturusleber arbeitete.

Diese Befunde sprechen für die stoffliche Natur der induktiv wirksamen Faktoren. Es bleibt aber ungeklärt, ob beim Induktionsvorgang ein Stoffaustausch zwischen Aktions- und Reaktionssystem stattfindet oder ob molekulare Veränderungen an den Grenzflächen genügen (Weiss, 1950), die Differenzierungstendenzen des Ektoderms zu realisieren. In beiden Fällen muß aber die Induktion der unterschiedlichen Komplexe abhängig sein von primär im Aktionssystem liegenden Differenzen. Wenn daher im folgenden von chemischen Stoffen gesprochen wird, die für den Induktionseffekt verantwortlich sein können, so sind damit nur die chemisch-physiologischen Faktoren des Induktionsmaterials gemeint, es soll aber nichts über den Wirkungsmechanismus ausgesagt werden.

In erster Linie hat Toivonen (1940, 1950, 1951) die Auffassung vertreten, daß für die Realisierung der Differenzierungstendenzen des undeterminierten Ektoderms mehrere qualitativ unterschiedliche Stoffe verantwortlich gemacht werden können. Lehmann (1950) hat ausgeführt, wie man sich aus dem Mischungsverhältnis von zwei Induktionsfaktoren, einem archencephal wirkenden und einem spinocaudal wirkenden, die regionalspezifischen Wirkungseffekte vorstellen kann. Es scheinen aber zwischen den Komponenten, die verantwortlich sind für die Realisation der einzelnen Komplexe kompliziertere Beziehungen zu bestehen, als bei einer einfachen Mischung der Induktionsfaktoren zu erwarten wäre.

In unseren Versuchen kam es bei Verwendung von Nierenrinde und Parotis als Induktionsmaterial sowohl nach Alkoholbehandlung der Implantate als auch nach Mangeldiät der Spendertiere einerseits zu einem Abfall der Zahl der induzierten C/D-Komplexe, andererseits zu einem fast spiegelbildlichen Anstieg der Zahl der A-Komplexe. Es ist die Frage zu klären, ob das antagonistische Verhalten auf die Beeinflussung nur eines Induktionsfaktors zurückgeführt werden kann oder ob sowohl die archencephal wirkende als auch die spinocaudal wirkende Komponente gleichzeitig betroffen werden. Die zuerst angeführte Möglichkeit scheint die größere Wahrscheinlichkeit für sich zu haben. Als Beweis dafür sollen die Ergebnisse der Leberserien herangezogen werden: Lebergewebe ist ein überwiegend archencephal wirkender Induktor mit verhältnismäßig schwacher Induktionsaktivität. Würde durch Alkoholbehandlung oder durch Mangeldiät die A-Komponente beeinflußt, müßte es in Parallele zu den Nierenexperimenten zu einer Veränderung, wahrscheinlich zu einer Steigerung der Zahl der induzierten archencephalen Komplexe kommen. Das war aber nicht der Fall. Aus diesen Befunden kann geschlossen werden, daß in der Nierenrinde und Parotis neben der spinocaudalen die archencephale Induktionskomponente mitenthalten ist. Sie kann aber nur dann wirksam werden, wenn durch chemisch-physiologische Veränderung des Aktionssystems dessen spinocaudale Leistung zum Verschwinden gebracht wurde. Der archencephal wirkende Faktor wird sowohl in der Nierenrinde und Parotis als auch im Lebergewebe und in der Herzmuskulatur durch die verschiedenen Manipulationen offenbar nicht betroffen.

In diesem Zusammenhang muß die Frage diskutiert werden, ob für die Realisation des deuterencephalen Komplexes ebenfalls eine spezifische Komponente im unterlagernden Material verantwortlich gemacht werden kann oder ob er durch das Zusammenwirken von A- und C/D-Faktoren hervorgebracht wird wie Lehmann (1950) vermutete. Bei der Prüfung der induzierten Komplexe ergab sich, daß in fast allen Fällen, in denen typisch spinocaudale Induktionsgebilde — in erster Linie komplette Schwänze — nachzuweisen waren, fast immer auch Deuterencephalon mit Ohrblasen gefunden wurde. Wir nehmen deshalb an, daß der deuterencephale Komplex ein Teil des C/D-Komplexes ist und auf der Wirkung desselben Induktionsfaktors beruht. Dafür spricht auch die Feststellung, daß bei der überwiegend archencephal induzierenden Herzmuskulatur nach langer Alkoholbehandlung die deuterencephale Leistung herabgesetzt wird, der B-Komplex sich demnach gegenüber Alkoholbehandlung genau so verhält wie der spinocaudale. Wir vermuten, daß die Realisationsmöglichkeit für deuterencephale Organe abhängig ist von der Stärke des spinocaudal wirkenden Faktors. Bei voller Wirksamkeit entstehen deuterencephale und spinocaudale, bei Abschwächung nur noch deuterencephale Organe. Mit dieser Annahme läßt sich auch die von Rotmann (1950) gefundene und durch unser eigenes Untersuchungsmaterial voll und ganz bestätigte regional-spezifische Anordnung der induzierten Komplexe erklären. Rotmann konnte zeigen, daß in einem Induktionskomplex archencephale und spinocaudale Organe nur dann zusammen auftreten, wenn auch deuterencephale vorhanden sind.

Aus unseren Versuchen lassen sich darüber hinaus noch Anhaltspunkte dafür gewinnen, daß auch innerhalb des spinocaudalen Komplexes Realisationsstufen in Abhängigkeit von der Stärke des Induktors bestehen. Bei Verwendung von Nierenrinde und Parotis als Induktionsmaterial kam es nach Mangeldiät der Spendertiere zu einer zahlenmäßigen Verminderung sämtlicher induzierter spinocaudaler Organe. Diese Abnahme erfolgte aber nicht gleichmäßig, sondern schon nach kurzer Hungerzeit waren die kompletten Schwänze sehr vermindert, während die übrigen spinocaudalen Gebilde erst nach länger dauernder Mangeldiät betroffen wurden.

Auf Grund der angeführten Befunde kann folgende Arbeitshypothese zur Erklärung der Leistungsspezifität der heterogenen Induktoren aufgestellt werden: Ein Induktor, der nur die archencephal wirksame Komponente enthält, wird archencephal induzieren; sind gleichzeitig auch spinocaudal wirkende Faktoren vorhanden, werden je nach der Stärke deuterencephale oder deuterencephale und spinocaudale Organe induziert. Die anscheinend immer vorhandene archencephale Induktionsfähigkeit kann mehr oder weniger verdeckt sein und häufig überhaupt nicht zur Wirkung kommen.

Auch mit der Auffassung von Nieuwkoop (1952) stehen die Befunde nicht in

Widerspruch. Bei einer Übertragung seiner Ansichten über den Induktionsvorgang auf unsere Experimente würde das besagen: Von den Nieren- und Parotisimplantaten geht ein 'transformierender' Einfluß aus, der durch Alkoholbehandlung oder Mangeldiät der Spendertiere ausgelöscht werden kann. Es bleibt dann nur das 'aktivierende' Prinzip übrig. Lebergewebe und Herzmuskulatur besitzen fast nur 'aktivierende' Tendenzen, die weder durch Alkoholeinwirkung noch durch Hunger der Spender beeinflußt werden können.

Nach Nieuwkoop's Ansicht besteht die 'Aktivierung' in einer Deblockade der archencephalen Differenzierungstendenzen, sie erfolgt immer, wenn ein bestimmter Schwellenwert erreicht ist, in voller Stärke. Es käme demnach dem Reaktionsmaterial das Primat bei der archencephalen Organdifferenzierung zu. Einige Resultate unserer Versuche mit heterogenen Induktoren lassen sich dagegen in der Richtung deuten, daß hier quantitative Verschiedenheiten des Induktionsmaterials für die Realisierung bestimmter archencephaler Organe verantwortlich sind — allerdings nicht in dem Sinne, daß sich spezifische Organinduktoren nachweisen ließen, wie Chuang (1939) und Toivonen (1945) vermutet haben. Betrachtet man im einzelnen die induzierten archencephalen Organe unter Berücksichtigung der Induktionsaktivität der betreffenden Serie als Maß für die Stärke des Induktors, dann ergibt sich, daß langfristig alkoholbehandelte Nierenrinde und Parotis als starke, überwiegend archencephal wirkende Induktoren in erster Linie Vorderhirnkomplexe, Augen und Nasen hervorzurufen vermögen, während in den Leberserien, die eine geringere Induktionsaktivität aufweisen, die Gehirne und Augen gegenüber den freien Linsen und Balancern in einem niedrigeren Prozentsatz auftreten. Den schwächsten Induktionseffekt zeigte Leber nach Ribonucleasebehandlung. Die Hirnkomplexe waren fast ganz verschwunden, die Naseninduktionen stark vermindert, freie Linsen und Balancer dagegen nur wenig beeinflußt. Auf Grund dieser Tatsache könnte man annehmen, daß bei den durch heterogene Induktoren hervorgerufenen Komplexen Realisationsstufen für die archencephalen Organe in Abhängigkeit von der Stärke des Induktors vorliegen.

Es muß aber auch eine zweite Erklärungsmöglichkeit in Betracht gezogen werden. In den Serien, bei denen eine niedrige Induktionsaktivität festgestellt wurde, wird in vielen Fällen der Schwellenwert für die 'Aktivierung' nicht erreicht. Es können keine Organe entstehen, die primär durch eine Deblockade des kompetenten Ektoderms realisiert würden. Bei den trotzdem noch auftretenden archencephalen Gebilden wie freien Linsen und Balancern, handelt es sich um sekundäre Induktionen, die in der Normogenese erst im alternden Ektoderm hervorgebracht werden. Sie könnten in den Experimenten mit heterogenen Induktoren nach Kompetenzeinschränkung des Reaktionsmaterials auch durch einen niedrigeren Schwellenwert des archencephal wirksamen Faktors hervorgerufen werden. Nach dieser Annahme wären die Differenzierungsmöglichkeiten im archencephalen Bereich nicht direkt von quantitativen oder qualitativen Veränderungen des Induktionsmaterials abhängig, sondern von der sich mit den

Kompetenzverhältnissen ändernden Schwellenempfindlichkeit des Reaktionsmaterials.

Die Experimente von Chuang (1939, 1940) und von Toivonen & Kuusi (1948) sowie von Yamada & Takata (1955) hatten die Proteinnatur des spinocaudal wirksamen Agens wahrscheinlich gemacht. Unsere Untersuchungen bringen dafür weiteres Beweismaterial. Länger dauernde Behandlung der Nierenrinde- und Parotisimplantate mit 70%igem Alkohol hatte ähnlich wie Hitze- oder Formoleinwirkung ein starkes Sinken der Zahl der induzierten spinocaudalen Organe zur Folge. Den gleichen Effekt rief Hunger bzw. Eiweißmangeldiät der Spendertiere hervor. Für beide Untersuchungsbefunde läßt sich wahrscheinlich machen, daß die Denaturierung der Proteine in den Implantaten durch die Alkoholbehandlung oder deren Verschwinden durch Eiweißmangeldiät für das genannte Ergebnis verantwortlich ist. Über die Form, in welcher die für den Induktionseffekt wirksamen Eiweißverbindungen vorliegen, kann nichts ausgesagt werden. Die in der Leber und Herzmuskulatur vorhandenen Proteine scheinen jedenfalls hinsichtlich der spinocaudalen Induktionsleistung nur wenig wirksam zu sein, während die Eiweißverbindungen der Nierenrinde und Parotis höchst aktiv sind. Daß auch im Urdarmdach hinsichtlich der Proteine ähnliche Verhältnisse vorliegen, wird durch die Experimente Rollhäuser's (1953) belegt. Schon eine sehr kurzfristige Denaturierung (5 min.) mit Alkohol hatte hier ein Verschwinden sämtlicher spinocaudaler Organe zur Folge.

H. Lehmann (1938) und Brachet (1942) hatten auf die Bedeutung der Nucleinsäuren beim Induktionsvorgang hingewiesen. Um die Vermutung, daß die Ribonucleinsäuren eine entscheidende Rolle bei der Realisierung des archencephalen Komplexes spielen zu beweisen, wurde von Kuusi (1951, 1953) eine Reihe von Experimenten ausgeführt, die aber kein eindeutiges Ergebnis brachten. Unsere eigenen Versuche, in denen durch Fermentbehandlung der Implantate versucht wurde, die archencephale Leistungsspezifität zu beeinflussen, führten ebenfalls nicht zu klaren Resultaten. Wie beschrieben kam es in den überwiegend spinocaudalen Nieren- und Parotisserien nach Ribonucleasebehandlung zu einer geringfügigen Erhöhung der archencephalen Rate. In den Serien, bei denen die Nierenrinde- und Parotisimplantate längerer Alkoholeinwirkung ausgesetzt waren und die dann eine archencephal-deuterencephale Spezifität zeigten, hatte die Fermentbehandlung fast keine Veränderung zur Folge. Während in den Hungerserien mit Parotisimplantaten die archencephale Leistung durch Ribonucleasebehandlung nicht beeinflußt werden konnte, wurde sie in den Nierenserien durch die Fermenteinwirkung herabgesetzt. In erster Linie scheinen die archencephalen Hirnteile betroffen zu sein. Diese Tendenz kam noch deutlicher zum Ausdruck in den Untersuchungen mit Lebergewebe nach Ribonucleasebehandlung. Hier waren primäre Induktionsgebilde wie Gehirn und Augen fast vollkommen verschwunden. Eine Auslöschung der neuralen Induktionsfähigkeit konnte aber nicht erreicht werden. Unsere Experimente erbringen damit keinen Beweis dafür, daß die Ribonucleinsäuren bei der Realisierung des

A-Komplexes von entscheidender Bedeutung sind. Sie lassen aber die Möglichkeit offen, daß sie eine akzessorische Rolle bei der Induktion des A-Komplexes oder einzelner Organe spielen können.

SUMMARY

1. With the use of the 'Einsteck' method the specific inducing abilities of several tissues of the guinea-pig were tested. Competent ectoderm of early gastrulae of *Triturus vulgaris* and *T. helveticus* served as reacting material.

2. After treatment with alcohol for a short period kidney (renal cortex) and parotid gland showed a deuterencephalic-spinocaudal, liver and heart-muscle an archencephalic inductive effect.

3. Increasing the duration of alcohol treatment (up to 20 days) changed the inducing abilities of kidney and parotid from deuterencephalic-spinocaudal to archencephalic-deuterencephalic. The same effect appeared after prolonged semi-starvation (3–4 weeks) of the donor animals. The archencephalic inductive effect of the liver and heart-muscle was not changed either by prolonged alcohol treatment of the implants or by starvation of the donor animals (only liver was tested). No dependence of inducing ability on age of donor animals was found.

4. These results permit the conclusion that there are two different modes of reaction in the competent ectoderm produced by the underlying action system. It seems that each inducing system contains the archencephalic component which can be dominated by deuterencephalic-spinocaudal inducing factors. If the latter should disappear through any change of the inducing material, e.g. a denaturation caused by alcohol treatment, archencephalic differentiation tendencies appear in every case.

5. The fact that induced structures of the deuterencephalic-spinocaudal complex decreased in number either as a consequence of alcohol treatment of the implants or through starvation of the donor animals indicates the importance of a degradation or decrease of proteins in the inductive material. The experiments do not support the assumption that the ribonucleic acids are of decisive importance in the realization of the archencephalic complex.

Unser besonderer Dank gilt Herrn Professor Dr. E. Rotmann†, der die Anregung zu den vorliegenden Untersuchungen gab. Nach dessen Tod wurden die Arbeiten gefördert durch Herrn Professor Dr. O. Kuhn, dem wir ebenfalls herzlich danken möchten.

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Composition of the Foetal Fluids of the Rabbit

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WITH ONE PLATE

INTRODUCTION

It is apparently not generally recognized that the allantoic fluid forms a significant part of the foetal fluids of the rabbit, and most writers on this subject have considered it to be absent or present only in insignificant amounts (Needham, 1931; Lell, 1931; Windle, 1940). The allantois is depicted in earlier morphological works on the rabbit placenta as moderately well developed but restricted in extent to the area of the placental cotyledons (Duval, 1892; Minot, 1889; Mossman, 1937). No studies on the composition of the allantoic fluid in the rabbit are known to the writers with the exception of a comment by Jacqué (1903–4) on the hypotonicity of the allantoic fluid relative to the amniotic fluid and foetal blood and to its low sodium chloride content and an observation by Paton *et al.* (1908–9) on the presence of fructose within it.

The allantoic fluid of the rabbit will be shown in this paper to be significant in amount and to have distinctive physical and chemical properties. Its presence coincides with the period of functional activity of the mesonephros as judged by anatomical and histochemical criteria. The chemical composition of the allantoic fluid, with special reference to electrolytes and nitrogenous substances, is also in favour of the concept of this fluid as a product of the foetal kidneys. The evidence for this will be briefly discussed.

MATERIAL AND METHODS

Pregnant rabbits at all stages of gestation after the 11th day were killed by intravenous nembutal, and the foetal fluids were removed by glass pipettes and measured. Foetuses were fixed in appropriate solutions and the urogenital apparatus later removed for histological study.

The protein of the fluids was estimated by a modification of Kingsley's method (1942). Urea nitrogen was estimated by the method of Natelson *et al.* (1951), uric acid by a modification of the Folin method (1933). The method of Natelson & Pennial (1955) was used for calcium determinations: inorganic phosphate was

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determined by the method of Fiske & Subbarow (1925). Sodium and potassium were estimated by the internal standard method employing the Baird flame spectrophotometer. Chloride was determined by the method of Schales & Schales (1941). Total reducing substance (TRS) was estimated by the method of Somogyi (1952). Non-fermentable reducing substance was estimated by fermentation with washed yeast. Fructose was estimated by the method of Roe (1934).

Determinations were always made in duplicate if the amount of fluid allowed it, but if the fluids from a single embryo were being studied this was not always possible.

RESULTS

Volume and appearance

The approximate volumes of the amniotic and allantoic fluids are shown in Table 1. These volumes varied with the age of the embryo and also from one embryo to another within a single litter. The figures are the average values obtained at each age. The amniotic fluid appeared first, at about the 11th day, the allantoic fluid not till the 13th day. The volume of the amniotic fluid was greatest from about the 20th to the 28th day and it had shrunk to nothing before term. The volume of the allantoic fluid was at its height about the 22nd day yet

TABLE 1

Volumes of the amniotic and allantoic fluids of the rabbit at different stages of gestation

Stage in mm.	*Age in days*	*Amniotic fluid in ml.*	*Allantoic fluid in ml.*
5	11	0·4	0
7·5	12	1·0	0
8	13	0·5	0
10	14	0·5–0·75	0·25
13	15	1·0	0·5
16	16	1·5	0·75 (op)
17	16	1·5	0·75 (op)
25	18	1·5	0·75 (op)
29	20	1·0	0·5 (op)
30	20	2–3	1·0 (op)
33	20	2–3	1–2 (op)
37	21	3–4	1·5 (op)
43	22	3–4	2–2·5 (op)
45	22	3·0	1·0 (m)
55	23	3·0 (a)	0·4 (m)
65	25	3·0 (a)	0·3 (m)
68	25	4·0 (a)	0
70	26	3·0 (a)	0
92	29	2·0 (a)	trace
95	29	0·5 (a)	trace
105	31	trace	trace 0·5

a = amber; op = opalescent; m = milky.

it disappeared so quickly after this time that it became difficult to identify it after the 24th day. Small amounts of milky allantoic fluid may occasionally be identified at term. Exocoelomic fluid could usually be collected from the exocoelomic space between the amnion and the overlying yolk sac. Its volume was sometimes similar to that of the amniotic fluid but it too was very variable and usually greatest when the embryos were dead and the amniotic sac correspondingly small.

Both the amniotic and allantoic fluids were clear in the early stages. At about the 18th day, however, the allantoic fluid characteristically became opalescent, and this easily differentiated it from the amniotic and exocoelomic fluids. By the 22nd day the allantoic fluid was milky while the amniotic fluid remained a clear amber colour (Table 1; Plate, fig. A). The opalescence and milkiness of the allantoic fluid were largely due to the presence of colloidal particles which undergo rapid brownian movement when observed under the microscope. The particles were removed if the fluids were shaken with ether or other fat solvents and centrifuged, so that they were probably fatty in nature and possibly analogous to the chylomicrons of the peripheral blood. The particles were too small to stain for identification with the usual fat dyes such as Sudan black or Scharlach R.

Composition of the foetal fluids

The composition of the amniotic, allantoic, and exocoelomic fluids and of the foetal blood-serum for four separate litters are shown in Table 2. The serum which was analysed was, in each case, a pooled sample from several foetuses; the fluids from individual foetuses were analysed separately and the results averaged. The amniotic fluid resembled the serum, although the calcium and chloride were somewhat higher and the potassium lower. The allantoic fluid differed strikingly

TABLE 2

Composition of amniotic, allantoic, and exocoelomic fluids, and of foetal blood-serum of the rabbit

Stage	*Fluid*	*Urea mg./100 ml.*	*Creatinine*	Ca *mg./100 ml.*	Na *m.eq./l.*	K *m.eq./l.*	Cl *m.eq./l.*
21 days	Amniotic				150	4·1	151
40 mm.	Allantoic				94	31·0	43
22 days	Amniotic	43	2·0		135	4·2	104
42 mm.	Allantoic	101	—		24	59·6	68
	Exocoelomic	34	1·7		129	4·8	98
24 days	Amniotic			10·1	137	6·3	117
60 mm.	Allantoic			—	72	43·0	83
	Serum			8·6	135	8·3	109
27 days	Amniotic			19·8	149	6·0	105
85 mm.	Serum			15·0	150	8·7	94

from the amniotic fluid and foetal serum in that the sodium and chlorides in it were lower and the potassium much higher. No allantoic fluid was found in the older (27-day) foetus. The similarity of the amniotic and exocoelomic fluid in general composition is also shown in this table.

No systematic variations were observed in the composition of the foetal fluids from one stage of gestation to another, so that it seems justifiable to put together the results obtained at different stages of gestation. Table 3 shows the average composition and range of variation from one gestation to another of foetal blood-serum, amniotic fluid, and allantoic fluid, taking all stages of gestation together. The products of a single pregnancy were pooled for analysis. No allantoic fluid can be obtained after about the 24th day of gestation, so all these results apply to stages up to the 24th day but not later.

TABLE 3

Average composition (with ranges) of foetal blood-serum and of amniotic and allantoic fluids of the rabbit

	Foetal serum	*Amniotic fluid*	*Allantoic fluid*
Total protein, mg./100 ml.	—	335 (260–410) (2)*	38 (1)*
Urea, mg./100 ml.	54 (1)*	43 (41–47) (6)	62 (47–100) (5)
Creatinine, mg./100 ml.	1·2 (1)	2·2 (1·2–4·8) (5)	4·3 (2·3–6·7) (5)
Uric acid, mg./100 ml.	1·3 (1)	1·2 (1·1–1·5) (3)	1·1 (0·8–1·7) (3)
Inorganic phosphate, mg./100 ml.	9·6 (1)	4·2 (2·8–5·9) (3)	4 (1)
Sodium, m.eq./l.	144 (135–150) (3)	145 (130–170) (8)	71 (24–90) (6)
Potassium, m.eq./l.	7·4 (5·2–8·7) (3)	6·3 (3·9–13·9) (8)	45·6 (35·7–64·7) (6)
Chloride, m.eq./l.	103 (84–109) (3)	112 (102–151) (7)	63 (42–83) (4)
Calcium, mg./100 ml.	12 (8·6–15) (3)	13·7 (10·1–19·8) (6)	22·2 (11·2–29·5) (3)
Total reducing substance, mg./100 ml.	56 (42–69) (17)	58 (45–104) (17)	78 (46–134) (17)
Non-fermentable reducing substance, mg./100 ml.	0	13 (9–18) (5)	28 (22–35) (5)
Fructose, mg./100 ml.	1·8 (1·0–3·3) (4)	3·9 (1·9–5·9) (4)	3·7 (2·0–5·9) (4)

* Numbers in parenthesis refer to number of gestations included in the average and range.

The values for each of the fluids showed considerable variation and it is possible to draw only general comparisons between them. Again it is seen that the amniotic fluid resembled the foetal serum more than did the allantoic fluid which was fundamentally dissimilar in its composition from both these fluids. The amniotic fluid contained much more protein than the allantoic. The allantoic fluid showed evidence of concentration above serum levels with respect to urea, creatinine, potassium, and calcium: it showed dilution with respect to sodium and chloride. The concentration of uric acid was about the same in both fluids as it was in serum. The total reducing substance of the allantoic fluid was higher than that of the amniotic fluid and both were lower than average serum values. There was, however, a higher percentage of non-fermentable reducing material in the allantoic fluid than in the amniotic fluid, so that the two fluids resembled each other more closely when allowance was made for this. The fructose content of both fluids was about the same and resembled that of serum.

The foetal kidneys

The mesonephros is quite rudimentary at the 11th day of gestation. By the 12th day the mesonephric (Wolffian) duct opens into the cloaca which, in turn, communicates with the cavity of the allantois. The latter reaches the surface of the placenta about the same time, and vascularization of the trophoblast by the allantoic mesenchyme begins. The mesonephros shows signs of functional activity after the 12th day and possesses well-developed glomeruli and tubules at least in the anterior half of the organ. The mesonephros is apparently fully active by the 17th day. Specimens examined contained about 40 nephrons on each side. The glomeruli were large and well vascularized, and easily visible to the naked eye. The tubules were coiled and, after corrosion of the mesonephros with concentrated acid, could be unravelled. The average length of each tubule was about 6 mm., of which about 4 mm. made up the proximal segment. There was no Henle's loop as in the metanephros, the transition from proximal to distal tubule being abrupt (Plate, figs. B, D). The characteristic cytology of the two segments is illustrated in fig. B. The distinctive features found in the proximal tubule were large amounts of fat at the base of the cells (Plate, fig. C), a strongly positive periodic acid-Schiff brush border (Plate, fig. D), and the presence of strong alkaline phosphatase activity in the free or luminal margin of the cells (Plate, fig. E). Degeneration of the mesonephros was well advanced in embryos of 22 days and was almost complete by the 24th day.

The metanephros, or definitive kidney, develops later than the mesonephros and is probably incapable of function before the 20th day at which time Gersh (1937) first observed the tubular elimination of dyes. The loop of Henle first appears in the metanephros about the 20th day. There is thus an overlap of function between the mesonephros and the metanephros from about the 20th to the 24th day after which the metanephros is the sole kidney.

The appearance of the allantoic fluid about the 13th day and its reduction and final disappearance after the 22nd day thus coincide well with the period of functional activity of the mesonephros as judged by anatomical and histological criteria.

DISCUSSION

The general similarity between the composition of the amniotic fluid and the foetal serum is not inconsistent with the concept of the origin of the fluid as a dialysate of the foetal serum, though definite evidence for this is not available. In a study of the human amniotic fluid Makepeace, Fremont-Smith, Dailey, & Carroll (1931) suggested that the fluid was at first derived from the foetal blood by dialysis and was later diluted by foetal urine. Cantarow, Stuckert, & Davis (1933) did not support this view and stressed the differences between the amniotic fluid and the foetal serum. All authors are agreed on the hypotonicity of the fluid at least in the later stages. No cryoscopic studies were made on the foetal fluids of the rabbit in this investigation, but these have been made by Dickerson & McCance (1957). In the latter third of gestation the amniotic fluid becomes discoloured and resembles urine, so possibly it is becoming diluted by foetal urine at that time. The urethra is patent as early as the 17th day so that there is no anatomical reason against this. The allantois probably constitutes the path of least resistance for the foetal urine until about the 24th day.

The close similarity between the composition of the exocoelomic fluid and that of the amniotic fluid suggests that the latter may be derived directly from the former. The exocoelomic fluid, however, may be derived independently but also by dialysis from the foetal blood in the rich vascular plexuses in the wall of the yolk sac.

The differences between the composition of the allantoic fluid and the foetal serum in the rabbit suggest the activity of a specific mechanism and rule out some simple concentrating device such as the abstraction of water across the allantoic wall. In favour of a renal origin for this fluid are the following observations: (1) the close correlation between the volume of the fluid and the onset and decline of function of the mesonephros as judged by histological criteria, (2) the anatomical continuity between the allantoic sac and the urogenital sinus or primitive bladder of the embryo, (3) the presence of colloidal fat in the allantoic fluid which may be correlated with the intracellular fat of the proximal mesonephric tubules, (4) the similarity between the composition of the allantoic fluid and that of foetal urine as far as this has been studied in the human foetus (McCance & Widdowson, 1953) and in the rat (Daly *et al.*, 1947). The latter point requires some amplifying. McCance & Widdowson (1953) have characterized the pre-natal urine of the human baby as hypotonic, low in sodium and chloride, very low in urea and potassium, and almost lacking in inorganic phosphate. Daly *et al.* (1947) found a more concentrated urine in the rat but with a very low level of sodium chloride and urea. It is not known if the allantoic fluid steadily accumulates or if there is a constant turnover of its constituents as in the amniotic fluid (Gellhorn & Flexner, 1942). Studies similar to those of Flexner and his school using isotopic methods supplemented by cryoscopic studies on the foetal fluids may give information of value in elucidating the origin of these fluids and renal activity in foetal mammalian life. The high potassium of the rabbit allantoic fluid is also a problem of provocative interest and is apparently an observation which has not been made on the allantoic fluid of other species.

SUMMARY

The composition of the foetal fluids of the rabbit at different stages of gestation has been described with reference to nitrogenous materials, electrolytes, total reducing substance, and fructose.

The composition of the amniotic and exocoelomic fluids resembles that of the foetal blood-serum except for the low total protein of the former. These findings

are consistent with the origin of these fluids as a dialysate of the foetal blood. The allantoic fluid is characteristically opalescent largely due to the presence of colloidal fat. Its composition also differs from that of the foregoing fluids. Thus the total protein is lower while the level of urea nitrogen and creatinine is higher. Sodium, chlorides, and inorganic phosphates are lower; calcium and potassium are higher. Total reducing substances are about the same in all three fluids when allowance is made for the higher non-fermentable reducing substance of the allantoic fluid: all are lower than the foetal blood-levels. The fructose content is low and about the same in all fluids.

The presence of allantoic fluid from about the 13th to the 24th day of gestation coincides with the period of function of the foetal mesonephros as judged by embryological and histological criteria. The structure of the nephron of the mesonephros is briefly described and the presence of alkaline phosphatase, fat, and periodic acid Schiff-reacting substances in the proximal tubules is illustrated. The evidence is consistent with the origin of the allantoic fluid as wholly or in part a product of the foetal mesonephros.

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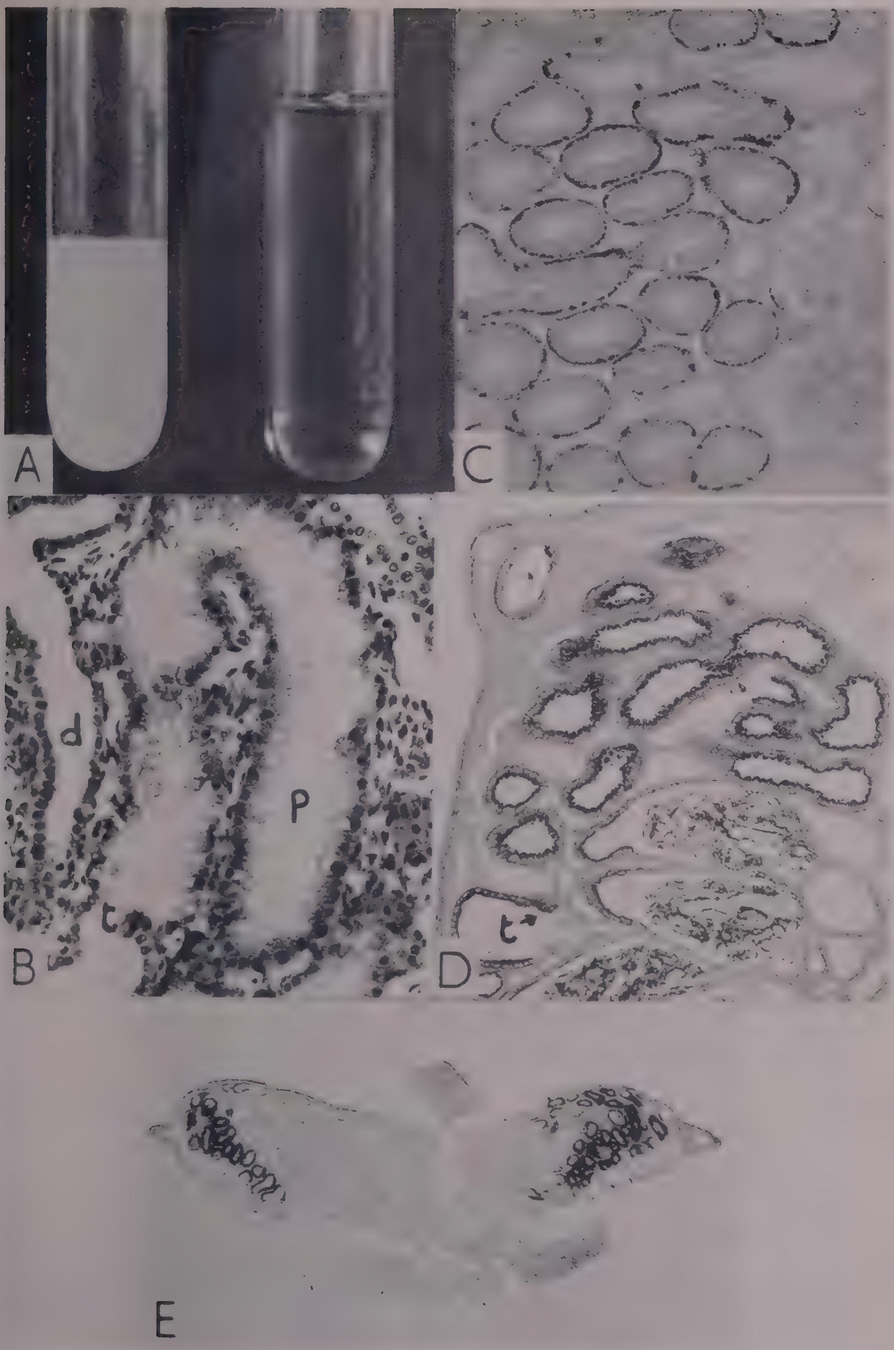

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EXPLANATION OF PLATE

FIG. A. Foetal fluids of rabbit at about the 21st day of gestation. The amniotic fluid (right) is clear while the allantoic fluid (left) is milky.

FIG. B. Mesonephric tubule of rabbit embryo of 17 days. The cells of the proximal tubule (p) are acidophilic and show marked apical vacuolation: those of the distal tubule (d) are cuboidal and are not vacuolated. The transition (t) from proximal to distal tubule is abrupt. There is no loop of Henle. Haematoxylin and eosin. ×225.

FIG. C. Section of mesonephros of rabbit embryo of 18 days showing discrete droplets of osmiophilic material in the basal part of the cells of the proximal tubules. Flemming's solution, paraffin embedding. ×120.

FIG. D. Section of mesonephros of rabbit at 17 days. The brush border of the proximal tubules stains intensely with the periodic acid-Schiff reagent as do the basement membranes of the glomeruli and intertubular sinusoids. Note the sudden loss of the brush border at the point of transition (t) from proximal to distal tubules. Periodic acid-Schiff, Bouin fixation. ×100.

FIG. E. Section of mesonephros of rabbit embryo of 17 days showing intense alkaline phosphatase activity in relation to the brush border of the proximal tubules. The glomeruli and distal tubules show insignificant enzymic activity. Absolute alcohol, Gomori technique, sodium glycerophosphate for one hour at pH of 9. ×25.

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The Composition and Origin of the Allantoic Fluid in the Rabbit

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INTRODUCTION

DAVIES & ROUTH (1957) have described the characteristics of the rabbit's allantoic fluid and shown that this contained much less sodium chloride than the foetal serum but considerably more potassium. Other properties such as the concentration of urea in the allantoic fluid and its temporal relationship to the mesonephros made them conclude that it was the produce of this organ. They did not make any measurements of the total osmolar concentrations of the fluids or sera. Owing to the kindness of these authors we have been able to make this measurement before their work was sent to press, and to consider their results in relation to those which we had already obtained in the pig and in man (McCance & Widdowson, 1954). This short note embodies our results and conclusions.

MATERIAL AND METHODS

Fluids were obtained from two rabbits 20 days after copulation. The parent animal was killed by a blow on the neck, and blood obtained for serum by heart-puncture. The uterus was removed from the body and transferred to a clean dissecting dish. On removing the uterine wall, the foetus surrounded by its sacs was exposed. Each fluid was obtained by puncturing the membrane and sucking out the fluid with a Pasteur pipette. When each sac was empty it was carefully dissected away in order to expose the succeeding one. The fluid was transferred to tared tubes and the amount obtained determined by weighing. Complete collections of the same fluid were pooled for analysis. Fluids were preserved under toluene.

Urea was determined by the method of Lee & Widdowson (1937); Cl by the method of Sendroy (1937); Na and K in the Beckman flame photometer. Total osmolar concentrations were obtained from the freezing-points which were determined by Hervey's (1955) procedure.

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RESULTS

There was no difficulty in confirming the essential findings of Davies & Routh. The opalescent appearance of the allantoic fluid was highly characteristic and distinctive. The table sets out the quantitative results which have been obtained. The concentrations of urea and potassium in the serum were lower than those in the allantoic fluid but the concentrations of sodium and chloride higher. Mainly owing to the latter the osmolar concentrations of the allantoic fluid were lower than that of the serum, but by no means so much lower as they have been found to be in the pig (McCance & Dickerson, 1956). The difference, as in the pig, was largely due to the relative concentrations of sodium and chloride in the serum and in the fluids.

Composition of the foetal fluids and of the maternal serum of the rabbit at 20 days' gestation

Litter	*Fluid*	*Average volume per foetus ml.*	*Urea mg./100 ml.*	Na *m. eq./l.*	K *m. eq./l.*	Cl *m. eq./l.*	*Total osmolar concentration m. mol/l.*
1	Amniotic	0·96	43·0	144	4·98	115	307
	Allantoic	0·38	59·8	108	43·0	80	304
	Maternal serum	—	41·3	—	—	112	333
2	Amniotic	1·58	36·6	144	6·7	110	312
	Allantoic	0·67	39·5	90·5	33·2	72·1	306
	Exocoelomic	3·29	37·2	152	6·2	105·2	312
	Uterine	—	—	121·5	22·0	—	310
	Maternal serum	—	32·5	138·6	—	107·6	328

DISCUSSION

If the conclusions of Davies & Routh (1957) are correct, and there seems no reason to doubt them, the allantoic fluid of the rabbit may be regarded as being mainly, if not entirely, the product of the mesonephros. This gives a chance of studying the function of this organ, and it would appear to separate a fluid which is hypotonic, due mainly to the concentrations of sodium and chloride in it being so much lower than they are in the serum. Present knowledge suggests that the foetal kidneys of all mammals do something similar, but naturally to very variable degrees. If this be so, some source for the 'free' water excreted by these foetal kidneys must be found. Aspects of this problem and its implications were discussed by McCance & Widdowson (1954), and in the light of their work on the pig it was suggested that the 'free' water was derived from the activities of the allantoic membranes and, later, the placenta itself. Further work is being carried out to prove or disprove this hypothesis.

SUMMARY

1. The allantoic fluid of the rabbit at the 20th day of gestation has been found: (*a*) to be opalescent; (*b*) to be hypotonic relative to the maternal serum; (*c*) to contain higher concentrations of urea and potassium than the maternal serum; and (*d*) to contain lower concentrations of sodium and chloride.

2. The facts known about this fluid indicate that it may be very largely a product of the foetal mesonephros.

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The Composition and Origin of the Foetal Fluids of the Pig

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MANY of the unicellular organisms have a greater concentration of osmotically active material within their body fluids than there is in the medium surrounding them, and the higher animals have glands like the kidney and sweat glands which are able to elaborate fluids with much lower concentrations of sodium and chloride than the body-fluids from which they were derived. The concentrations of sodium and chloride in hypotonic urine and sweat are much lower than those in the serum, and consequently the total osmolar concentrations are also lower, but the urea is characteristically higher. There is no proof that the process is one involving the secretion of water; and, indeed, the kidney is now thought to produce a hypotonic urine by the active reabsorption of sodium from the distal tubule, the walls of which are impermeable to water unless there is posterior pituitary hormone in the circulation.

The foetal fluids and membranes deserve more consideration in this connexion than they have hitherto received. Jacqué (1903–4, 1905–6) pointed out that the allantoic fluid of the sheep was hypotonic and propounded the theory that this fluid was largely foetal urine which he also demonstrated to be hypotonic. This idea has been brought up to date by McDougall (1949) and by Alexander, Nixon, Widdas, & Wohlzogen (1955 *a*, *b*). Paton, Watson, & Kerr (1908–9) discussed the whole question of the origin of foetal fluids—human and otherwise—in the light of the knowledge which existed at that time. There is no doubt, as they pointed out, that these fluids are of foetal origin, but the progress of knowledge as to their nature and origin has always been obscured by (i) the great variability in their volume and composition even between littermates and certainly with the progress of gestation, (ii) obvious species differences, (iii) a tendency to compare their composition with foetal urine, rather than with foetal or maternal serum, (iv) a failure to appreciate the importance of their total osmolar concentration, and (v) neglecting to search for general principles and generalizations.

Davies & Routh (1957) have recently made out a strong case for regarding the allantoic fluid of the rabbit as the product of the foetal mesonephros. Their essential findings have been confirmed (Dickerson & McCance, 1957) and the

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fluid shown to be slightly hypotonic to the maternal serum. Wislocki (1935) made an elaborate study of the volume of allantoic fluid and of amniotic fluid in the pig and cat. The volume relationships in the two species were not the same. Wislocki drew attention to the fact that the allantoic fluid of a pig foetus might exceed 200 ml. before the embryo was 3 cm. long and added that 'this circumstance in the sow cannot be reconciled with the theory of the allantoic fluid being in whole or major part an excretory product of the mesonephros'.

McCance & Widdowson (1953) pointed out that there were grounds for believing that the foetal kidney of the sheep and man, and probably of other species, constantly produced a very hypotonic urine in relatively large amounts and that this must involve the secretion of water by the placenta into the foetus or the removal of sodium and chloride from it. They suggested (McCance & Widdowson, 1954) that the pig's allantoic membrane carried out the same function while the placenta was still undeveloped, and that this accounted for the low osmolar concentration found by them in the pig's allantoic sac about the 24th day of pregnancy. Some further work has now been done on the composition of the allantoic and amniotic fluids of the pig.

MATERIAL AND METHODS

Eleven cross-bred Large White × Wessex or Essex sows were used to provide material for these studies. They were killed at the slaughterhouse 20–65 days after service. The uterus was removed as soon as possible after death and in most cases a sample of maternal blood was also collected. The uterus and blood were taken without delay to the laboratory where the serum was separated and the uterus dissected. Each foetus was transferred to a porcelain dish and samples of allantoic fluid, and in later stages also of amniotic fiuid, were withdrawn with a syringe for analysis. The volume of fluid withdrawn was noted and the volume still remaining in the sac was then determined by rupturing the sac and pouring the fluid into a measuring cylinder. Samples of fluid for analysis were preserved under toluene.

The physical and chemical methods used in the analysis of the fluids were those given by Dickerson & McCance (1957) with the additional estimation of creatinine and phosphorus by modifications of the methods described by Hawk, Oser, & Summerson (1947).

RESULTS

1. *The effect of age*

Table 1 shows the composition of the allantoic fluid at several stages of gestation. In many ways the results are incomplete but the experiments were ranging ones and the important and suggestive findings will be elaborated later. The points to note are: (1) at 20 days the volume was small, the total osmolar concentration 175–290 m.osmols/l., with an average value of 256, and the concentration of urea in the fluid nearly always lower than that in the maternal body-fluids; the concentration of creatinine was always lower. The uterine fluids, which may perhaps be regarded as the medium surrounding the sow's foetus at this age, were analysed on two occasions. The concentrations of creatinine in them were found on both occasions to be the same as those in the allantoic fluids and the concentrations of urea to be the same on one occasion and slightly higher on the other. The figures obtained were: for creatinine on the first occasion 1·3 and 1·4 mg./100 ml. for the two fluids and on the second occasion 1·5 and 1·5 mg./100 ml. respectively; and for urea, on the first occasion 2·6 and 2·7 m.mol./l. and on the other 3·45 and 3·1 m.mol./l. respectively. As in serum the concentration of sodium ions in the allantoic fluids exceeded that of chloride ions. (2) At 41–46 days the volumes were larger, as Wislocki had shown, and the total osmolar concentrations considerably lower, for at this age all were below 150 m.osmols/l. whereas at 20 days they were all above. The concentration of urea in the fluids was higher than in the sow's body-water and the concentration of creatinine

TABLE 1

The effect of age on the composition of the allantoic fluid

		20 Days		*41–46 Days*		*55–65 Days*
		Fluid	*Sow's serum*	*Fluid*	*Sow's serum*	*Fluid*
No. of fluids analysed:		32	3	16	2	5
No. of litters:		3	—	2	—	3
Volume (ml.) . . .	Average	5·3	—	110	—	353
	Range	1·4–10·7	—	80–200	—	82–727
Total osmolar concentration (m.osm./l. of water) . .	Average	256	317	120	313	92
	Range	175–290	306, 327	86–146	302, 324	70–120
Urea (m.mol./l. of water) .	Average	3·1	3·7	8·4	5·3	10·3
	Range	2·2–4·3	3·1–4·1	6·2–9·8	4·7, 6·1	8·6–11·9
Creatinine (mg./100 ml. of water)	Average	1·2	2·1	3·7	1·8	—
	Range	0·8–1·7	1·8–2·3	1·7–5·8	1·7, 1·9	—
P (mg./100 ml. of water) .	Average	8·6	8·5	6·6	6·9	—
	Range	6·1–13·2	7·9–9·8	4·8–11·7	6·4, 7·3	—
Cl (m.eq./l. of water . .	Average	69	111	30	113	18
	Range	35–94	108–114	17–47	110, 116	15–24
Na (m.eq./l. of water) . .	Average	114	148	13	145	14
	Range	62–134	146–151	3·4–32	137, 153	4·8–20
K (m.eq./l. of water) . .	Average	14·3	7·6	8·2	6·6	6·0
	Range	9·6–20	7·2–8·2	3·3–16	5·4, 7·8	1·1–12·6

chromogens considerably higher. The fall in the total osmolar concentration was largely due to the fall in the concentration of sodium ions. The potassium did not fall to the same extent and neither did the chloride, and at this age the chloride ions frequently exceeded the sum of the sodium and potassium ions. The essential findings shown at this age are all confirmations of results obtained on three other litters which had been analysed previously but less completely. The limited observations made on foetuses aged 55–65 days take the changes a stage further than those noticeable at 46 days. Although sows' serum was not analysed at this age, it may be assumed that its composition remains approximately constant throughout pregnancy. The average volume of allantoic fluid was considerably higher than at 41–46 days and the osmolar concentration lower. The percentage of sodium and chloride in the fluids had fallen farther and that of urea had risen a little. The concentration of chloride ions did not always exceed that of sodium and potassium at this age but it was always greater than that of sodium alone.

2. *The effects of volume*

Table 2 shows the composition of the largest and smallest volumes of allantoic fluid in three litters. It is to be noted here that: (1) there were great variations in volume; (2) this did not materially alter the concentration of urea within the fluids; but (3) it made a considerable difference to the concentrations of electrolytes, and although the osmolar concentrations were not measured, values for the minerals show that the larger volumes had lower ones. Age, however, was probably more important in defining composition than volume.

TABLE 2

The effect of volume on the composition of the allantoic fluid

	42 Days		*43 Days*		*44 Days*	
Volume (ml.)	54·0	2·0	22·0	3·5	59·0	1·5
Urea (m.mol./l.) . . .	7·8	7·3	7·7	7·9	7·9	7·1
Cl (m.eq./l.)	33·2	70·5	27·0	48·7	22·5	80·0
Na (m.eq./l.) . . .	—	—	19·9	45·5	—	—
K (m.eq./l.)	—	—	3·0	6·5	—	—

3. *The amniotic fluid*

Table 3 shows results which have been obtained on the amniotic fluid. At the two later age periods its composition relative to allantoic fluid may be seen by referring to Table 1. The more gradual increase in the volume of this fluid, as compared with that of allantoic fluid, is again in agreement with the findings of Wislocki (1935). Its volume at 46 days averaged only 26 ml. The total osmolar concentration was always over 260 m.osmols/l. In man, Makepeace, Fremont-Smith, Dailey, & Carroll (1931) suggested that the amniotic fluid may be isotonic with maternal serum early in gestation and be diluted later with foetal urine. In the pig we have found no evidence of isotonicity with maternal serum but the possibility of the amniotic fluid being isotonic with foetal serum at some stage has not been excluded; indeed, the one value obtained for the total osmolar concentration of foetal serum at 46 days was the same as the average figure for the total osmolar concentration in the amniotic fluids at this age, but there was a considerable scatter in the osmolar concentrations among the individual fluids. On the two occasions when maternal serum and amniotic fluid were analysed side by side, the concentrations of urea, creatinine, and phosphorus were lower in the amniotic fluid than in the maternal body-fluids. The concentration of sodium and chloride ions approximated to those found in the maternal body-water. The concentration of potassium ions was higher in the amniotic fluid than in maternal serum and at 46 days had the same average value as the cord plasma, which was considerably higher than the figure for the maternal serum (Widdowson & McCance, 1956). The fluids obtained in the period 55-65 days had a lower concentration of potassium ions than those at 46 days.

TABLE 3

The effect of age on the composition of the amniotic fluid

		34 Days	*41–46 Days*		*55–65 Days*
		Fluid	*Fluid*	*Sow's serum*	*Fluid*
No. of samples:		1	13	2	5
No. of litters:		1	2	—	3
Volume (ml.)	Average	—	26	—	62
	Range	—	22–31	—	49–82
Total osmolar concentration (m.osm./l.)	Average	296	290	313	294
	Range	—	263–301	302, 324	274–322
Urea (m.mol./l.)	Average	3·0	4·5	5·3	4·8
	Range	—	2·9–5·0	4·7, 6·1	4·0–5·9
Creatinine (mg./100 ml.)	Average	—	0·8	1·8	0·3
	Range	—	2·6–1·0	1·7, 1·9	—
P (mg./100 ml.)	Average	4·0	4·1	6·9	6·5
	Range	—	3·2–6·0	6.4, 7·3	5·8–7·2
Cl (m.eq./l.)	Average	103	104	113	92
	Range	—	86–107	110, 116	92–93
Na (m.eq./l.)	Average	125	126	145	119
	Range	—	117–135	137, 153	116–121
K (m.eq./l.)	Average	21	18·9	6·6	9·3
	Range	—	11·5–22·7	5.4, 7·8	7·4–10·4

DISCUSSION

The variability in the composition and volume of the pig's allantoic fluids with age is at first confusing, but the work carried out on them up to date makes it legitimate to put forward the following statements about them. (1) Their composition relative to that of the maternal or foetal sera shows that they are certainly formed by a process of secretion rather than dialysis. (2) The allantoic fluids have initially a small volume and an osmolar concentration not far removed from that of the serum. Between the 20th and 41st day they increase greatly in volume and decrease considerably in osmolar concentration. The latter change is due mainly to a fall in the concentration of sodium ions and to a lesser extent of chloride ions, but there is also a fall in the concentration of potassium. There must be some mechanism in their formative cells for secreting free water into the foetal fluids or for removing sodium, potassium, and chloride from them. The latter seems more probable. The effects of these mechanisms become progressively more conspicuous between the 46th and 65th day of gestation. Attempts were made to demonstrate the secretion of water and (or) the reabsorption of sodium by the allantoic membranes *in vitro*, but these were not successful in a saline phosphate medium (Robinson, 1949) whether glucose and glutamic acid had been added or not. (3) The fluids are often of a size relative to the foetus which makes it very difficult, if not impossible, to regard them as products of the kidney (Wislocki, 1935; McCance & Widdowson, 1954). They must in all probability be formed by the allantoic membranes themselves, although their composition may be modified by the activities of the foetus. The physiological interrelationship between these formative cells of the allantoic fluids and the water metabolism of the foetus has still to be explored. Great species differences must be expected. (4) The concentration of urea at 20 days is usually below that of the maternal serum but not that of the uterine secretions. By the 46th day the concentration in the fluids is higher than that found in the maternal or foetal body-water. As in the formation of sweat, therefore, water must be removed at some stage or urea added. The latter is perhaps more probable but there is no evidence one way or the other. These statements are true also of the creatinine chromogens. If the changes in volume are taken into account it is clear that the fluids contain much more urea and creatinine chromogens at the latter than at the former date, and since the concentration of urea in the fluids has by 41–46 days risen above that in the foetal or maternal serum, the escape of urea from the fluids must be less rapid than it is across the placental barrier. The source of this additional urea and creatinine chromogen may be the foetal urine but this remains to be proved.

Judging by their volume and composition the origins of the amniotic fluids must differ from those of the allantoic fluids. In early pregnancy the amniotic fluids increase relatively slowly in volume and change little in their total osmolar concentration. The fact that at this age the concentrations of urea and creatinine chromogens in the fluids are below those in the maternal or foetal serum speaks against their having a renal origin. It also suggests that some secretory mechanism must be involved although the concentrations of electrolytes are of the order to be expected in an ultrafiltrate or dialysate.

SUMMARY

The foetal fluids of the pig have been investigated at 20, 46, and 65 days after mating.

(1) The allantoic fluid has been found:

- (*a*) to increase rapidly in volume and decrease in tonicity between 20 and 65 days. The decrease in tonicity was largely due to the fall in the concentration of sodium ions;
- (*b*) to contain lower concentrations of urea and creatinine at 20 days than the parent body-fluids, but to contain higher concentrations at 46 days;
- (*c*) at 20 days to contain, like serum, an excess of sodium ions over chloride ions but at and after 46 days always to contain an excess of chloride ions over sodium ions and sometimes over sodium + potassium;
- (*d*) to differ greatly in volume from one foetus to another even within the same uterus. This difference in volume was often accompanied by considerable differences in the concentrations of electrolyte *but not in that of urea.*

These findings suggest that the allantoic fluid may be produced initially by a secretion of the allantoic membranes and that its composition may be modified later by the metabolic products of the foetus.

(2) The amniotic fluid has been found:

- (*a*) to increase relatively slowly in volume during early pregnancy;
- (*b*) to be somewhat hypotonic to the maternal body-fluids during the whole of its existence and at 46 days to have lower concentrations of creatinine and urea;
- (*c*) to contain concentrations of sodium and chloride similar to those in maternal or cord serum;
- (*d*) to contain concentrations of potassium approximating to those in the foetal serum.

We would like to thank Mr. R. W. Pomeroy for his never failing co-operation and Terry Cowan for his care in managing the animals.

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Tracer Studies on Chick Embryos *in vitro*

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WITH TWO PLATES

INTRODUCTION

In the experiments to be reported here isotopic tracer techniques were combined with the techniques of chick embryo culture *in vitro* and of organizer transplantations. The distribution of methionine taken up under these conditions was first tested by autoradiography; methionine was also used to label organizer grafts and observations were made on the derivatives from such grafts; finally, the transfer of labelled molecules from the grafts to the induced structures was to some extent investigated.

The first use of radioactive tracers to investigate the transfer of substances in induction was by Waddington (1950), employing P^{32} on Amphibian embryos. Our experiments can be viewed as an extension of these investigations and those of Abercrombie & Causey (1950) and Islam (1953) who first used radioactive tracers to label embryonic grafts in chick embryos and explants respectively, and of Feldman & Waddington's (1955) work on the uptake of labelled methionine by the chick embryo *in ovo*.

MATERIALS AND METHODS

Chick embryo cultures

These were set up according to the ring technique described by New (1955). This consists essentially in stretching the explanted blastoderm under a glass ring in a watch-glass. As a nutritive medium 0·8 ml. of liquid egg albumen is added underneath the blastoderm. A few drops of Pannet and Compton's solution are placed on the upper surface of the blastoderm. The watch-glass is kept in a Petri dish lined with wet cotton wool. In the present experiments the embryos transferred to culture were all at the definite primitive streak stage (18–20 hours of incubation) and the cultures were incubated at 38·5° C. for a further 22–24 hours before fixation. They will live under these culture conditions for much longer but we wished to investigate the distribution of tracer before it had spread too widely.

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Labelled methionine

This was DL-methionine-S^{35} supplied by the Radiochemical Centre, Amersham. Its specific activity varied in different batches from 11·8 to 19·9 mc./mM.

Autoradiographic technique

The blastoderms were fixed in acetic alcohol and sectioned at 6 μ. Autoradiographs were obtained by the stripping film technique of Doniach & Pelc (1950) with an exposure of 5–7 days.

EXPERIMENTS AND RESULTS

I. *Methionine uptake* in vitro

Embryo cultures were set up and incubated as described. Radioactive methionine was mixed with the albumen used as culture medium, in the proportion of 2·5 μc. per ml. The embryos were found to be, at fixation, in the 10–14 somite stages.

The examination of the autoradiographs led to the following findings: (i) The neural tube and the notochord show a higher energy uptake than any other structure. The very highest activity is in the dorsal wall of the neural tube and adjacent areas of the ectoderm (Plate 1, fig. A). (ii) Whilst the head mesenchyme and the endoderm show a much lower level of activity, the somites and the myoepicardium have activities nearly as high as the neural tube. The high activity of the myoepicardium contrasts with the low activity of the rest of the mesodermal splanchnopleura and is already very marked when the paired heart primordia are still widely separated (Plate 1, fig. A).

II. *Grafting of an unlabelled organizer*

In this experiment a Hensen's node from a donor blastoderm of the same age was grafted into each blastoderm *in vitro*. Following Waddington (1932), the organizer was inserted between the ectoderm and endoderm in the area pellucida of the host blastoderm and at the level of the host node. At fixation an induced neural tube was present in most cultures and, in addition, some self-differentiation of the graft itself had occurred. Out of eleven operated blastoderms, seven had developed an induced neural plate or tube.

The question at issue in this experiment was whether the host, the induced, and the graft neural tubes would take up the isotope at the same or at different rates. The autoradiographs showed that the rate of uptake was identical for all three neural tubes and mesoderms. In a few sections of two (out of twelve) specimens the induced neural tube had in some regions a markedly lower activity than the host. Since, however, this difference was only observed in some sections and not even in all sections on one slide, it was not considered significant.

III. *Grafting of labelled organizer*

In this experiment no labelled methionine was added to the medium, but the organizer grafts were labelled. The donor blastoderms were of the same stage as the hosts and had been labelled by the injection into the egg, prior to incubation, of a quantity of the isotope carrying about 10 μc. of activity. The injection was made in the manner described by Francis & Winnick (1953).

Examples of the autoradiographs obtained are given in Plate 1, figs. B, C, D. It will be seen that the tissues derived from the graft were clearly identifiable by their high activity. This activity was over 30 grains per unit area of 54 μ^2, against a background of only 3 to 5 grains.

The mesenchyme under the induced neural tissue appeared to be derived mainly, if not entirely, from the graft. The boundary between labelled and host mesodermal tissue was sharp. It is remarkable that in several cases a notochord attached to the induced neural tube is shown actually to be derived from the graft. Tissue from the graft was also responsible for the formation of the foregut lining accompanying the induced axis in one case. Plate 1, fig. D illustrates this specimen; the formation of the foregut wall by graft tissue is clearly seen and this wall is continuous with, but sharply distinguishable from, the wall of the host foregut.

The induced neural plate or tube showed a substantial activity uptake, in marked contrast to all tissues of the host axis (Table 1). The concentration of grains per unit area in the neural tube was not uniform throughout the neural tissue, but it was higher where this tissue was in contact with graft tissues. The impression given was of a diffusion gradient.

Two of the specimens are perhaps worthy of separate description:

The specimen of Plate 1, fig. B has both the induced and host neural tubes well developed. The graft is undifferentiated and is distinctly marked by its high activity. The notochord and the mesenchyme associated with the induced neural tube have a high activity concentration and appear to be derived from migrating graft tissues rather than from host tissues. If they were derived from host tissues which had subsequently taken up the labelled molecules it would be difficult to explain their high level of activity, their sharp demarcation from the host tissue, and the lack of a gradient of concentration in the mesenchyme.

Figs. C and D of Plate 1 represent sections at different levels of another specimen. In both figures some self-differentiation of the graft is obvious. At the level of the first figure the graft has formed a neural tube (open) with its notochord as well as a notochord attached to the induced tube. But it does not seem to have provided any mesenchyme. Again, the grain counts in the induced neural tube show a gradient. From A to B for example, the counts fall off in the following manner: 12–12–10–10–10–8–7–7–7–5–3–5 where each count is the average of four unit areas counted.

At a more posterior level (that of fig. D), the graft again seems to have produced no mesenchyme but it has instead differentiated into a wall of the foregut on the side of the induced neural tube. This wall is continuous with that of the host foregut and the demarcation is sharply defined.

The derivation of mesoderm from a graft is illustrated also by the specimen of

Plate 2, fig. E. The part of the graft which in this section is found outside the blastoderm shows no marked difference in the intensity of labelling from mesoderm accompanying the induced tube. This mesoderm is not in close contact with the neural tube and it is perhaps for this reason that the latter gives small grain counts.

IV. *Labelled organizer grafts plus unlabelled methionine*

Labelled Hensen node organizers were grafted as in experiments II and III. In addition 0·6 mg. of unlabelled free L-methionine was added per 10 c.c. of albumen of the culture medium. Four specimens were obtained, two of which showed induction. The results are illustrated in Plate 2, fig. F and Table 1. The activity uptake of the induced and host neural tubes was negligible in all specimens, except for some small areas lying in close contact with the graft.

TABLE 1

Counts above background per unit area of 54 μ^2 on autoradiographs of transverse sections of operated blastoderms. Each series of counts was made on a single section of one blastoderm and each entry is an average of 5 to 24 measurements, depending on the area in section of the structure examined

Neural tube		*Notochord*		*Mesoderm*		*Endoderm*		
Host	*Ind.*	*Host*	*Graft-derived*	*Host*	*Graft-derived*	*Host*	*Graft-derived*	*Remarks*
Experiment III: Labelled Organizer Graft								
6·2	29·3	10·0	..	2·0	9·5	9	40+	Figs. C, D
3·0	11·7	..	60+	2·0	10·0	..	..	
2·8	6·9	2·0	26·2	2·0	20·9	..	..	Fig. B
1·0	5·0	1·0	..	1·5	25·0	..	..	Fig. E
0·0	5·0	..	..	0·0	30·0	..	..	
Experiment IV: Dilution								
0·0	1·0	..	0·0	0·0	15·0	..	..	Note (*a*)
0·0	..	0·0	..	0·0	0·0	..	..	,, (*b*)
1·0	1·0	..	..	0·0	11·0	..	..	,, (*c*)
2·5	..	1·5	..	1·0	..	..	..	,, (*d*)

NOTES: (*a*) Graft also self differentiated.
(*b*) No induction. Graft counts: 15.
(*c*) A small area of the induced tube which is in contact with the graft showed an activity of 4 counts per unit area.
(*d*) No induction. Host neural tube in a section adjacent to the graft showed an activity of 9·2 counts per unit area.

V. *Labelled peripheral organizer grafts*

In this case small pieces of tissue from the area pellucida at some distance from the primitive streak of labelled donors were used as grafts. Four specimens were sectioned and autoradiographed. In two of these the graft had remained far from the axis of the host. It showed, of course, high activity and some self-differentiation. No uptake whatsoever by the host could be detected. The other two specimens are illustrated in Plate 2, figs. G, H. In the specimen of fig. G the graft has not caused induction but has self-differentiated into neural tissue. This graft was taken from the area on the left of and near the primitive streak and included presumptive neural tissue. The host neural tube has a substantial activity concentration on the side adjacent to the graft, but practically none on the opposite side. This is evidence of diffusion, occurring independent of induction.

The graft of fig. H has self-differentiated and also caused induction. This graft was derived from the area in front of and near the primitive streak of the donor and also included presumptive neural tissue. This area is still within the limits of the organizer region, the exact extent of which is under investigation (Mulherkar, unpublished). The induced neural tube gave counts from 10 grains per unit area at the base to 5 at the distant ends, whilst the host tube showed hardly any activity uptake.

DISCUSSION

The uptake of tracer by embryos cultivated in vitro

The study of methionine uptake *in ovo* by injection of labelled methionine into the egg as applied by Feldman & Waddington (1955) involved the injection into each egg of a quantity of the isotope carrying 5 μc. of activity, at a stage when the viability of the injected egg cannot be ascertained.

In view of the cost of isotopes, it is an advantage of the *in vitro* procedure that a smaller quantity need be used and that the viability of the blastoderm can first be checked. In experiment I of the present series only 2 μc. per culture were used and this quantity proved actually to be more than sufficient to label the embryonic tissues clearly. But the main advantage of the *in vitro* procedure is, of course, that it allows operations to be carried out on the explant and thus makes possible experiments such as those of the present series. Furthermore, the embryos *in vitro* can be accurately staged and periodically observed.

The sequence of relative activity uptake by the neural tube, notochord, somites, and endoderm was shown in experiment I to be similar to that found in the *in ovo* studies of Feldman & Waddington. The same authors suggest that the higher uptake in the upper parts of the neural fold might be connected with the production of the neural crest. The picture obtained in the stages studied in our experiment I (closed neural tube), where the area of highest uptake extends on either side of the roof of the neural tube, seems to confirm their hypothesis. The high activity of the myoepicardium shown in experiments I and II is of interest in connexion with early myosin synthesis. Ebert *et al*. (1955) suggested the use of tracer methods for the purpose of obtaining an index of myosin synthesis. Such an index would be necessary for the study of the mechanism underlying the gradual restriction of myosin-forming areas during early embryogenesis.

Comparison of induced and host embryos

The second experiment provided the opportunity to compare the methionine uptake and thus, presumably, the rate of protein synthesis of three neural tissue units (host, induction, and self-differentiating graft) developing in the same blastoderm. The fact that there is no rate difference may be attributed either to the genetic uniformity of these units or to their development under a single metabolic pacemaker, presumably the host organizer. The possibility of a common factor governing host and induction has been discussed by Waddington (1940) in connexion with the equality of mitotic rates in these two units. The neural plate often differentiating from the graft itself seems also to come under the same pacemaker, so far as methionine uptake is concerned.

It might prove possible in further experiments to test the relative roles of genetic constitution *versus* a common overruling factor by grafting *duck* organizer on to *chick* blastoderm and comparing the rate of uptake by the three units.

In any case, the confirmation of an equal rate of synthesis of host and induction raises two points: firstly, at the time of grafting the host axis is laid down already as a definite primitive streak, but the cells of the area pellucida which will give the induced neural tube are undifferentiated. During the following 24 hours, two neural tubes of the same degree of cellular differentiation arise. The one is, so to say, 48 and the other 24 hours old, but the rate of synthesis and the mitotic rate are in both equal. This is not surprising, as Feldman & Waddington (1955) have already noted that at early stages there is no difference in the uptake of labelled methionine between the primitive streak itself and the rest of the blastoderm. It appears that the increased protein synthesis connected with the neural tissue differentiation begins at about the stage when our graftings were made (24 hours of incubation) and thus both the induced and the host tissues differentiate in step.

Secondly, the higher uptake by the induced as opposed to the host tube shown in experiments II and V must be due to reasons other than higher synthetic activity. It might be due only to the position of the induced tissues near the labelled graft, or to that as well as to some selective transfer from the graft to the induced tissues.

Molecule transfer and induction

The question can be asked whether this transfer consists in a simple diffusion or in the transfer of a macromolecule which might have some causal connexion to the induction process. Experiments IV and V were undertaken with these questions in mind. In the former, in which unlabelled methionine was added to the culture as a 'trap', the activity uptake by the induced neural tissue is practically obliterated, except for areas directly opposed to the graft. This is evidence for diffusion of free molecules of the labelled amino-acid. It also proves that the transferred unit is the amino-acid molecule and not the labelled radical only. This evidence is strengthened by experiment V; it is shown in this that there is a marked transfer from a labelled graft to the neighbouring neural tube, irrespective of whether an induction process is taking place or not.

The conclusion seems inevitable, therefore, that induction is not correlated to a transfer of methionine-containing macromolecules from the graft on any large scale. But, as Waddington & Sirlin (1955) pointed out, this does not exclude the transfer from the graft to the inductively activated cells of a few molecules able to trigger off a chain reaction. If the results of these experiments provide no positive answer to the question of the nature of the inducing factor, they exclude at least the possibility of a large-scale transfer of macromolecules, and perhaps make more likely the alternative of the transfer of *small* quantities of the effective agent.

Derivation of mesoderm and notochord of the induced axis

The origin of the mesodermal structures accompanying the induced axis was uncertain and 'one must guess that they are formed partly from the original streak and partly by "induction"' (Waddington, 1956). Experiment IV leaves little doubt that the mesenchyme is in the main, if not entirely, and the notochord entirely, derived from the inducing graft. It is also shown that the graft can supply endodermal structures, namely the wall of the foregut, associated with the induced axis.

SUMMARY

1. Chick blastoderms of the definite primitive streak stage were cultured *in vitro* for about 24 hours with radioactive DL-methionine-S^{35} added to the medium. It was shown by autoradiography that the neural tube and especially its roof and also adjacent areas of the ectoderm (neural crest) take up more activity than other structures. Mesodermal structures such as the notochord, somites, and myoepicardium also reach a high level of uptake, presumably correlated with their differentiation.

2. Tissues of the host, the induced axis, and self-differentiating (non-labelled) grafts take up activity from the culture medium at the same rate.

3. If a labelled organizer is grafted on to a host blastoderm, the induced neural tube takes up a substantial amount of labelled molecules but the host does not. Experiments involving dilution with unlabelled methionine or grafting of labelled peripheral organizer tissue, produced evidence for the view that the activity is carried to the induced tissues by diffusion or exchange of free amino-acid molecules; transfer of macromolecules does not occur on a scale detectable by the methods used but it cannot be excluded that it might occur on a smaller scale.

4. In cases of induction caused by organizer graft the notochord, mesenchyme, and, occasionally, tissues lining the foregut associated with the induced axis are contributed by the graft.

We wish to thank Professor C. H. Waddington for his encouragement and generous help in our work and for the facilities provided at his department. We are indebted to the Melville Trust (E. P.) and to the Indian Government (L. M.) for a fellowship and an overseas scholarship, during the tenure of which this work was performed.

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EXPLANATION OF PLATES

PLATE 1

Abbreviations: *end.*, endoderm; *g.*, graft; *g. nch.*, notochord of the graft; *g.n.t.*, neural tissue of the graft; *h. nch.*, notochord of the host; *h.n.t.*, host neural tissue; *i. nch.*, notochord of the induction; *i.f.*, foregut of the induction; *i.n.t.*, neural tissue of the induction; *m.*, mesoderm; *my.*, myoepicardium.

FIG. A. Autoradiograph of a transverse section of a 48-hour embryo cultured *in vitro* for 24 hours, with tracer added to the medium.

FIG. B. Autoradiograph of a transverse section through a blastoderm including a labelled graft and an induced and the host embryos.

FIG. C. Autoradiograph of a transverse section of a blastoderm containing a self-differentiated labelled graft and the induced and the host neural tissues.

FIG. D. Autoradiograph of a transverse section of a blastoderm containing a labelled graft (which produced foregut wall) and the induced and host embryos.

PLATE 2

FIG. E. Autoradiograph of a transverse section of an induced embryo and the labelled graft. The host axis is not included in the photograph.

FIG. F. Autoradiograph of a transverse section of a blastoderm containing a labelled graft and the induced and host neural tubes. Non-labelled methionine was added to the medium.

FIG. G. Autoradiograph of a transverse section of a blastoderm in which the labelled graft

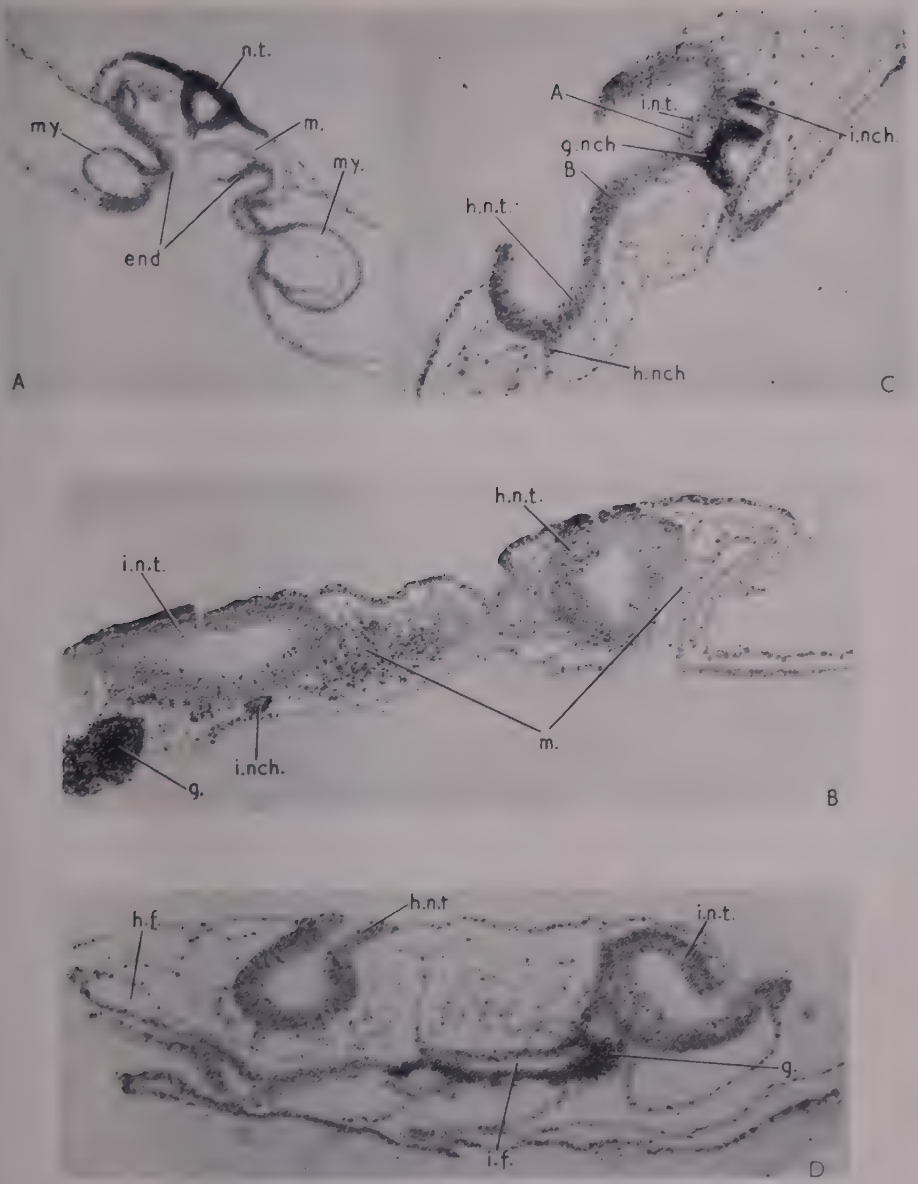

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Plate 1

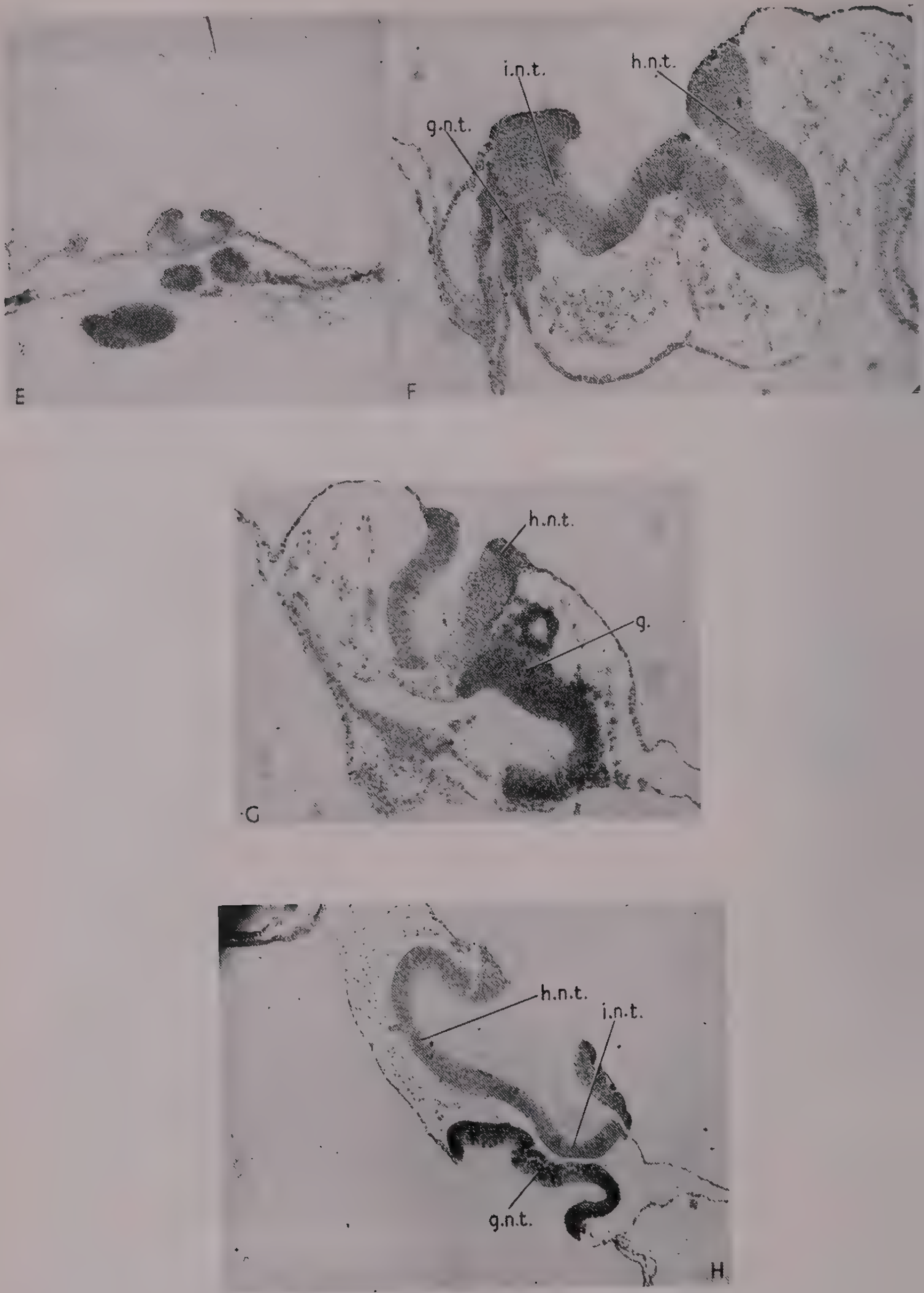

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Plate 2

caused no induction but shows self-differentiation and continuity with the host endoderm. Non-labelled free methionine was added to the medium.

FIG. H. Autoradiograph through a transverse section of a blastoderm with a labelled peripheral organizer graft which has caused induction and has also undergone self-differentiation. Non-labelled free methionine was added to the medium.

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On the Regulation of Bilateral Symmetry in Plutei with Exchanged Meridional Halves and in Giant Plutei

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From the Zoological Institute, Uppsala

INTRODUCTION

THE determination of bilateral symmetry in the sea-urchin egg has been the subject of investigations which have yielded contradictory results. The earlier literature was reviewed in 1936 (Hörstadius & Wolsky). It may therefore suffice to mention only the following points in this paper. The problem of whether or not the sperm induces the bilateral symmetry in the sea-urchin egg has not been solved by direct experimentation. By means of local vital staining opposite the point of sperm entrance Hörstadius (1928) concluded that there was no relation between the entrance point of the sperm and the position of the first furrow. The stain applied at the moment of fertilization soon became so diffuse that it was impossible to recognize the point of staining in late stages. It was at that time considered that the first furrow generally cut through the egg in either the median or the frontal plane, and only comparatively seldom obliquely to these planes (von Ubisch, 1925; Runnström, 1925; Hörstadius, 1928). As the sperm was found to enter at any spot between these two planes of preference, it was concluded that the sperm had no influence on the origin of the bilateral symmetry. However, the last-mentioned author did not consider his own or the earlier vital staining experiments to be sufficiently accurate. A new investigation with refined technique proved that the first furrow bears no relation to the median or frontal planes (Hörstadius & Wolsky, 1936). As the first furrow is independent of the entrance point of the sperm, and as the furrow has no relation to the median plane, the possibility that the sperm induces the bilateral symmetry could not be excluded.

Several authors have found that larvae derived from isolated blastomeres of the 2-cell stage show defects on the side that was considered to have faced the other blastomere. A systematic study of this point was undertaken by Hörstadius & Wolsky (1936). Thy reared isolated halves in pairs after vital staining of the cut side. It was found that right and left halves retain their dorso-ventral

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axis and are often slightly delayed or defective in their differentiation on the operated side. Ventral halves also develop their ventral field on the presumptive ventral side of the egg. But in dorsal halves the dorso-ventral axis is reversed. Oblique halves give intermediate results. The experiments were extended to meridional halves of unfertilized eggs. Within these pairs also some corresponding differences in development were found, indicating a bilateral constitution of the egg even before fertilization.

Driesch (1892, 1906) and Boveri (1907) isolated the blastomeres of the 4-cell stage and obtained dwarf plutei of typical shape. Hörstadius & Wolsky (1936) proved that all four $\frac{1}{4}$-blastomeres of one egg can develop into well-proportioned plutei. But in early stages one or two of them, evidently the dorsal ones, were delayed in their differentiation. The same authors also divided 32-cell stages into eight equal meridional fragments and found that some of them gave minute plutei. In the others the bilateral symmetry was more or less disturbed.

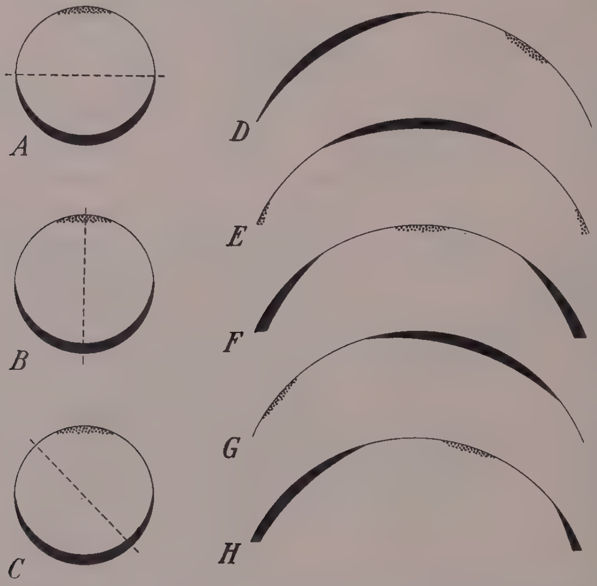
In many batches of eggs of *Paracentrotus lividus* a red pigment ring can be seen below the equator. Boveri (1901) and Lindahl (1932) studied eggs that had been stretched more or less perpendicularly to the animal-vegetal axis. The former noticed in three cases that the bilateral symmetry of the larva had been adjusted to the bilateral form of the stretched egg. Lindahl found that a more pronounced elongation had a remarkable effect. Two ventral fields appeared at the ends of the stretched egg. He suggests that in the normal egg one side has a stronger capacity to form a ventral side than other parts and that this ventral centre suppresses the tendencies of the same kind in other parts of the egg. In eggs sufficiently stretched one part would get beyond the reach of the subduing power of the stronger region and therefore be able to assert itself. The same explanation also holds for the reversal of the dorso-ventral axis in dorsal halves. When the influence of the presumptive ventral centre is eliminated the point least suppressed and therefore most likely to start the establishment of another ventral side is the point most distant from the dominating centre. Similar results were obtained with meridionally constricted eggs (Hörstadius, 1938). Oral fields also developed at the most distant sides if the one partner had no archenteron.

The investigation was carried out with eggs from *P. lividus* Lk. at Station Biologique, Roscoff, in the summers of 1950, 1951, and 1954. I am greatly indebted to the authorities of the station for accommodation and assistance.

RESULTS

Exchange of meridional halves between two eggs

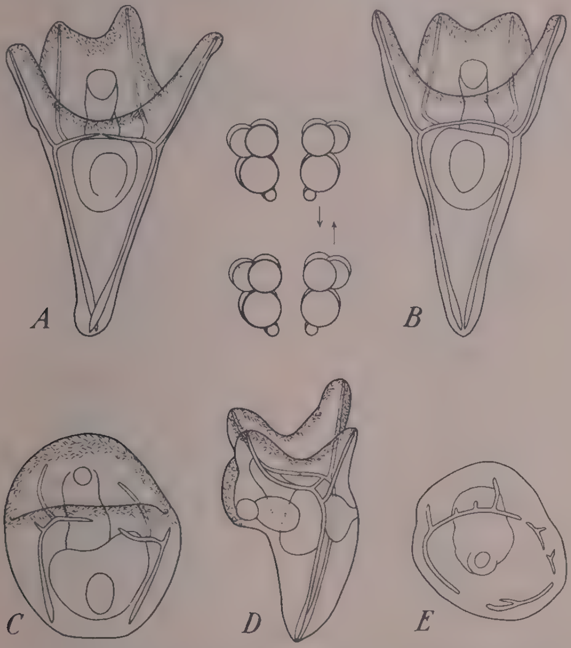
As the first and second cleavage furrows are independent of the presumptive bilateral symmetry, meridional halves isolated in the 16-cell stage may represent dorsal and ventral, left and right, or oblique meridional halves of the egg. In these fragments we postulate the presence of a ventral centre, decreasing towards left and right (black in Text-fig. 1 A–C), and a dorsal region where the suppressing influence of the ventral centre is weakest (dotted area). In oblique halves the plane of separation can, of course, have any position between the median and the frontal planes. If we transplant a meridional half of one egg to a meridional half of another egg with the same animal-vegetal orientation we can obtain every conceivable combination of the types of halves diagrammatically illustrated in Text-fig. 1 A–C. In a number of cases transplantations were made in such a way that in pairs of eggs the meridional halves were mutually exchanged (Text-fig. 2).



Text-fig. 1. Diagrams of eggs with a supposed dominating ventral centre (black) decreasing in a dorsal direction. The least inhibited material (dotted area) will be found at the presumptive dorsal pole. A–C: constitution of dorsal and ventral, left and right, and oblique halves. D–H: constitution of flattened whole cleavage stages cut open along radii corresponding to dotted lines in A–C.

At the time of operation we, of course, do not know anything of the constitution of the fragments. What is the power of regulation within these systems, the halves of which have been brought together at random?

In forty-three cases both partners of the pair successfully continued their development. In no less than forty-two of these pairs the bilateral symmetry was perfect, as illustrated by the pair shown in Text-fig. 2 A, B. The difference which occurred in a few pairs between the two plutei concerned not the pattern of bilateral symmetry but the speed of differentiation or an inhibition of outgrowth of the arms (Text-fig. 2C). Only in one of the forty-three pairs were the larvae clearly irregular. One of these larvae had a typical pluteus skeleton although the axes of the two halves at the time of fusion happened to form an angle to each other. As a result the invagination of the archenteron started at two nearby points, its tip became bilobed, and two oesophagi were formed (Text-fig. 2D). In this larva a short stretch of ciliated band had developed in a median dorsal position, as seen in Text-fig. 2D. The significance of this will be discussed in the following section. The other larva was of a radial type with several spicules in a ring round the base of the archenteron (Text-fig. 2E).

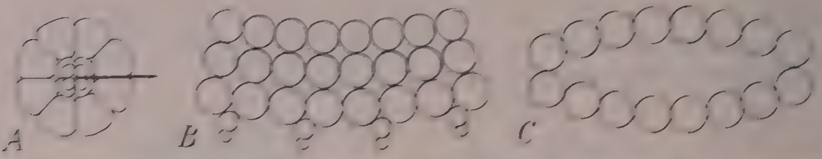


TEXT-FIG. 2. Exchange of meridional halves between two eggs. A, B: a pair of typical plutei. C: pluteus with typical bilateral symmetry but without arms. D: pluteus with two oesophagi and a supernumerary ciliated band on part of the dorsal side. E: irregular larva.

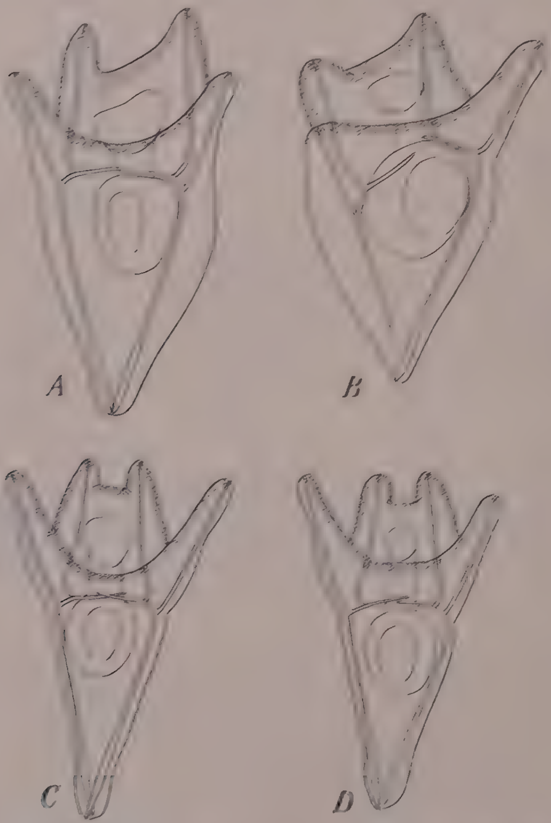
Fusion of two eggs

Several investigators have described fusion of sea-urchin eggs but only with random orientation with regard to their axes. The following is a study of giant larvae resulting from transplantations of two 32-cell stages in such a way that they acquire a common animal-vegetal axis.

Eggs in the 16-cell stage were put into Ca-free sea-water. After the subsequent cleavage division an egg was placed on its animal pole. With the tip of a glass needle a meridional cut was made from the centre between the micromeres (Text-fig. 3A). The 32-cell stage was then unrolled and flattened out as in Text-fig. 3B. The same operation was performed on a second egg. In the meantime the first egg often more or less closed itself up again and therefore had to be flattened out anew. After working alternately in this way on the two eggs it was possible to get them sufficiently flattened to put them into a furrow in a celluloid plate and



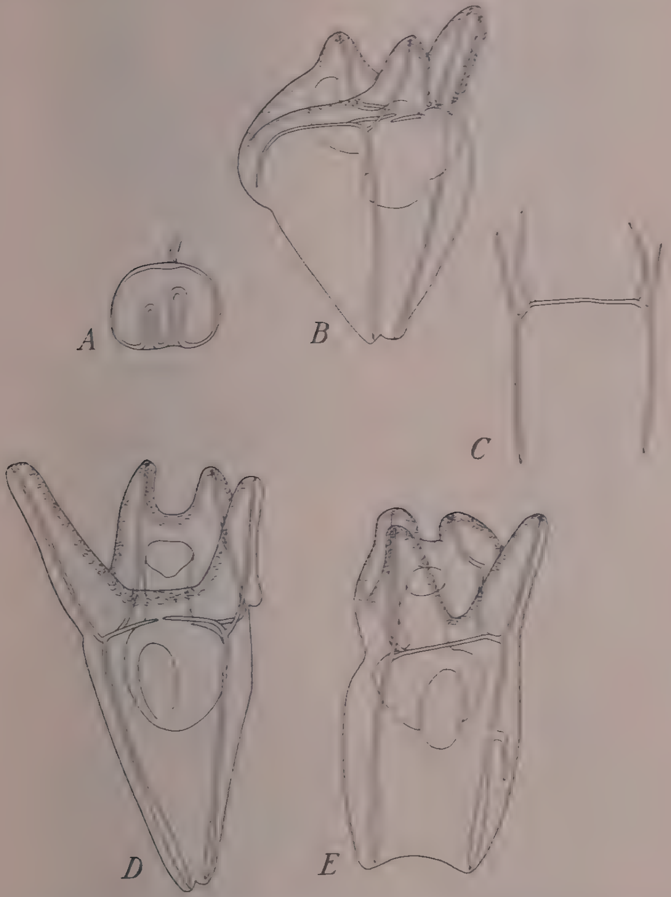
TEXT-FIG. 3. Fusion of two 32-cell stages to form a giant larva with unitary animal-vegetal axis. A: the egg is placed on its animal pole and cut along a meridian from the vegetal to the animal pole (thick line). B: the 32-cell stage flattened as one layer. C: one flattened egg placed on top of another, as seen from their animal ends.



TEXT-FIG. 4. Two giant plutei, and two normal plutei for comparison.

to press them together with a glass capillary. They were oriented with their egg axes parallel and with the same polarity. Text-fig. 3C shows diagrammatically the one egg placed on top of the other, both seen from the animal pole. Only the most animal row of the descendants of the mesomeres (the an₁-cells) are drawn.

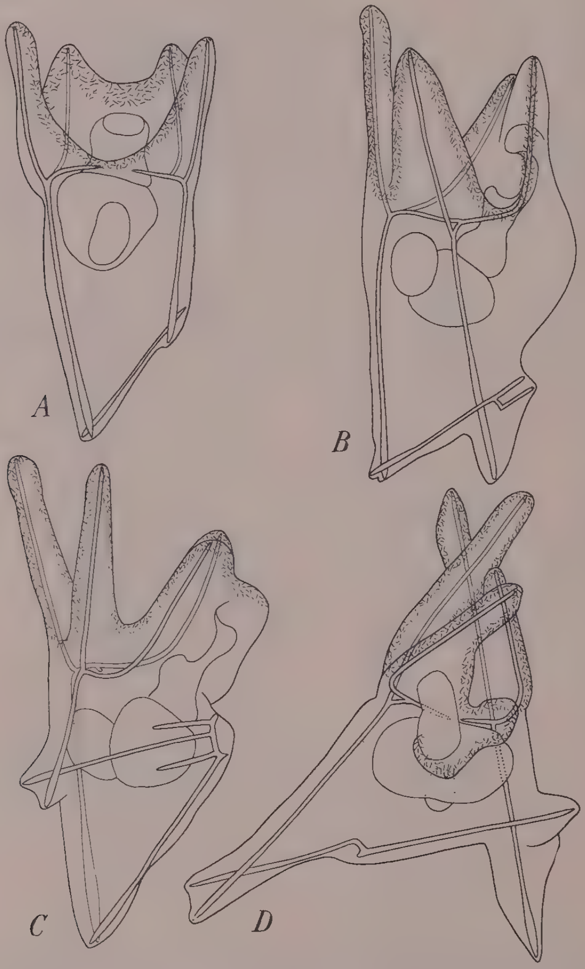
The giant blastula formed as a result of this transplantation has a comparatively short egg axis. It is, of course, actually of the normal length; but the circumference is twice as large as in a normal egg and, moreover, not circular but elongated, as seen in Text-fig. 3. As a consequence the vegetal material where invagination will start is not concentrated at a vegetal point but is extended along the elongated vegetal side. It sometimes happened, therefore, that invagination started at two points, leading to the formation of two archenterons (Text-fig. 5A). As the invagination proceeded the two tubes always fused at the base so that the



TEXT-FIG. 5. A: two invaginations in a giant gastrula. B–E: giant larvae. B: slightly atypical pluteus. C: skeleton of typical pluteus. D: typical pluteus. E: slightly atypical pluteus.

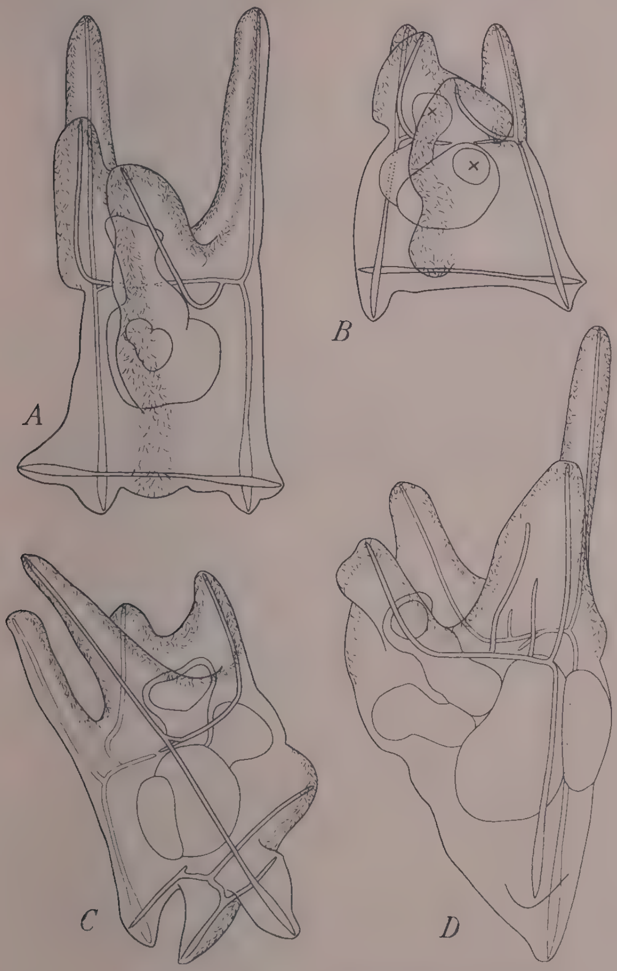
intestine in all cases and the stomach in most cases were unitary whereas the oesophagi in many larvae were double.

The giant larvae in several cases gave plutei of typical shape as seen from Text-fig. 4 where two control plutei are also drawn to show the difference in size. Another case of a perfect giant pluteus is presented in Text-fig. 5D. The skeleton C was found in the surface film. This larva had also been typical. In Text-fig. 5 B, E we find only small imperfections of the skeleton. It is evident, however, that the regulation has led to bilateral symmetry in all main points.



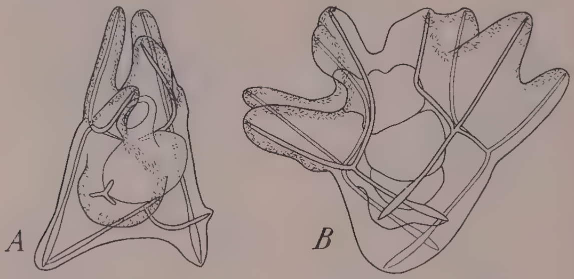
Text-fig. 6. Giant plutei with supernumerary dorsal skeleton rod.

The four larvae in Text-fig. 6 all have one trait in common. They possess a supernumerary piece of skeleton in the pointed part of the body which represents an elongated part of the dorsal side. This 'dorsal' rod expands this part of the body-wall and forces the body rods apart. Text-fig. 6D illustrates an extreme case where the divergence of the two halves of the skeleton at this part has caused the oral and anal arms of left and right side to cross. In C the dorsal skeleton has developed a number of branches.



TEXT-FIG. 7. Giant plutei. A–C: plutei with supernumerary dorsal rod and ciliated band and also with two oesophagi. D: pluteus with two oesophagi and piece of dorsal ciliated band (cf. Text-fig. 2D).

The plutei of Text-fig. 7 A, B also carry a dorsal rod but have another supernumerary differentiation. A strip of ciliated band runs more or less in the median line from the back of the oral lobe towards the dorsal spicule. In these two larvae we find two oesophagi, one larger and one smaller (in 7B both are marked with *X*). A similar strip of unpaired ciliated band can likewise be observed in the pluteus in Text-fig. 7D, which also has two oesophagi but no dorsal rod. It might

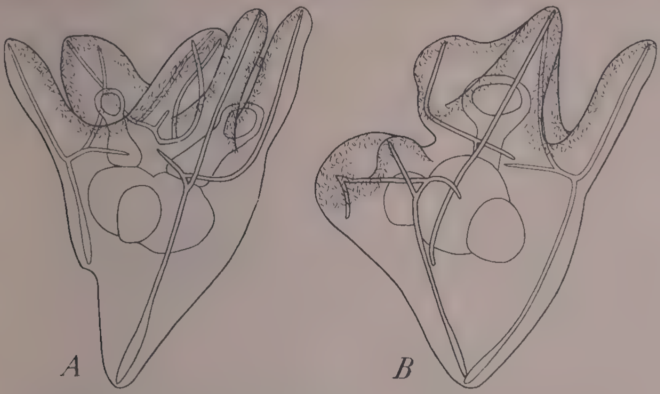


TEXT-FIG. 8. Giant larvae. A: pluteus with stronger tendency to form a second ventral side in the dorsal part; two pieces of skeleton, and a loop of ciliated band. B: double larva with two oral fields and two pairs of skeletons but only one stomodaeum.

seem from this as if the unpaired dorsal ciliated band has some connexion with the second oesophagus. That, however, is probably not the case. In Text-figs. 6D and 8A we also find an additional dorsal loop of the band but in larvae with only a single digestive tract. Moreover, in the pluteus of Text-fig. 7C the unpaired dorsal band has detached itself from the ciliated band of the oral field and is situated in the most dorsal part of the body, in close connexion with a dorsal rod.

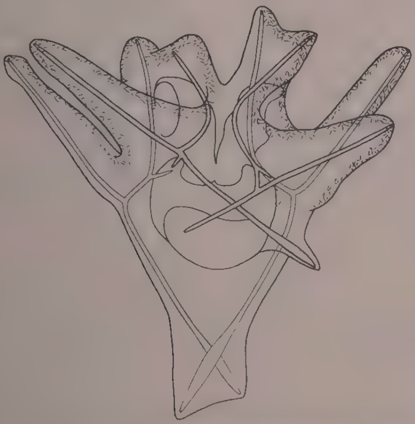
We can now say that the appearance of a dorsal rod or band is a first sign of a tendency to form a second ventral side. A second step is to have both rod and band. The latter in the pluteus of Text-fig. 7C has even developed at a distance from the oral field, and in the larva of Text-fig. 8A has made a coil as if to encircle a second ventral field. In this last case a second 'dorsal' triradiate spicule has also been formed, and the larger dorsal piece of skeleton resembles a normal one, with rods that perhaps correspond to oral, anal, and body rods. We note that there is only a single digestive tract in this larva.

A further step towards formation of two ventral sides in a giant larva is shown in Text-fig. 9A. There are two oesophagi, and the upper part of the stomach is slightly divided. I could not decide whether or not the ciliated band is continuous or is split into two separate bands. The skeleton consists of three pieces. One left and one right piece are perfectly typical, except that the body-rod of the right piece is unusually short. The third piece acts as right skeleton to the left and as left skeleton to the right of the joined twins formed by the digestive tract. There seems to be one oral and one anal rod for each of the twins. A body-rod has, of course, not been able to grow out as the stomach blocks the way. It is replaced by a short median rod which, however, has grown in a distal direction.



Text-fig. 9. Two joined twins each with stomach and part of the skeleton in common.

In the larvae of Text-fig. 9B we find two separate oral fields although only one stomodaeum. The partner with a mouth also has a better-developed skeleton. The middle piece has differentiated like the right skeleton of the left partner.



Text-fig. 10. Double larva with two oral fields, two pairs of skeletons, two oesophagi, and two mouths.

In Text-fig. 10 we see a case with a nearly complete doubling of the bilateral symmetry. There is only one intestine and one stomach, but two oesophagi, although they are fused at their bases. Two ciliated bands encircle two oral fields with mouths facing opposite directions. The two pairs of skeletons possess all the rods they should have, but the body-rods have grown to meet a body-rod of the other partner instead of the rod belonging to the same pair.

In the double larva of Text-fig. 8B the skeletons are still more perfect in their bilateral symmetry. There is only one mouth and one oesophagus. The latter, however, bears signs of having had a slightly bipartite origin. In spite of the lack of a second oesophagus and a stomodaeum there is a complete oral field developed in the part of the larva which in the gastrula stage was most distant from the rudiment of the other oral field.

DISCUSSION

As mentioned in the introduction, the sea-urchin egg has a bilateral organization even before fertilization. The basis of this constitution has been discussed by several authors. Schleip (1929, p. 537) advanced the hypothesis that the most vegetal properties might be eccentrically situated with regard to the egg axis. A similar opinion was expressed by Runnström (1931), i.e. that the vegetal gradient is stronger on the ventral side. This would establish a bilateral organization. These assumptions were tested by a series of isolation experiments (Hörstadius & Wolsky, 1936, p. 95). No trace of a preponderance of vegetal characters was found in the one or two isolated meridional quarters or parts of quarters which ought to have been particularly sensitive to slight changes in the animal-vegetal balance. In his paper on stretched eggs Lindahl (1932) discussed two possibilities. One hypothesis is that the animal and vegetal gradients do not run obliquely in relation to each other but that on the presumptive ventral side they both decrease more slowly than on the other side. Both properties would therefore be more strongly represented ventrally. According to this hypothesis, the gradients in dorsal halves would also be more strongly represented in their presumptive most ventral parts, thereby leading to differentiation without change of direction of the dorso-ventral polarity. However, this does not conform to the fact later revealed that in reality an inversion of polarity does take place in dorsal halves and the dorso-ventral axis is reversed.

Although there are several observations recorded in the literature which speak in favour of an asymmetrical arrangement of the animal and vegetal gradients as a cause of bilateral symmetry (see Lindahl, 1932; Hörstadius & Wolsky, 1936, p. 108), the appearance of radial symmetry under certain conditions also seems to speak against such an assumption. Herbst (1893, 1903) and Fischel (1909) obtained radially symmetrical larvae by the action of Li, K, Na, Ca, or Mg chlorides and other substances. It is hardly conceivable that an agent could so easily straighten an oblique gradient or weaken a double animal-vegetal gradient system along certain meridians but not others. If the suppression of the bilateral symmetry were a result of a change in the animal-vegetal system only, one should expect a noticeable shift of differentiation in the animal or vegetal direction in radially symmetric larvae. Such differences have in fact been observed, but both ways. Hörstadius & Gustafson (1954) state that in most series treated with 8-chloro-xanthine the apical tuft of the gastrula was somewhat enlarged, but, on the other hand, an unusually large archenteron was found in another series of radially symmetrical larvae.

It seems to me more plausible to apply the other hypothesis mentioned in the introduction. Every meridional part has the power to develop a ventral side, as $\frac{1}{4}$- and even $\frac{1}{8}$-larvae are able to develop into plutei. But a presumptive ventral centre suppresses these tendencies in the other parts of the egg. The results described in this paper agree with this assumption. When stretching eggs in a capillary pipette Lindahl (1932) found that the leading end became the ventral side. But if the metabolism of this end was retarded by too intensive local vital staining, the other end developed the ventral side. Similarly, Pease (1941, 1942 *a*, *b*) showed that after local application of many inhibiting substances the inhibited part of the egg produced the dorsal side. It seems to me likely that this dominating, metabolically active centre, easily inhibited by many substances, is of another nature than the animal-vegetal properties, although it is not precluded that its position in eggs with a deranged animal-vegetal system may depend on the new distribution of these properties.

When aspirating eggs through a narrow pipette Lindahl (1932) observed surface granules moving backwards from the front end towards the hind end. As the former formed the ventral side, the latter the dorsal side, one might suspect that displacement of material in connexion with stretching may be one of the reasons for the inversion of the dorso-ventral axis and that the same also happens when blastomeres are separated by a glass needle. This cannot, however, be the case. To be sure, the axis is reversed in dorsal halves after operation, but, on the other hand, the axes are not shifted in left and right halves. If the operation had the supposed effect, all cut surfaces ought to develop as dorsal sides. Moreover, the needle passes so easily between the cells, particularly when Ca-free sea-water is used, that any considerable effect on the cell cortex is improbable. Furthermore, if the cut surface were predisposed to develop as the dorsal side, we should not have obtained perfect plutei after the fusion of two halves (Text-fig. 2).

We saw in the introduction that left and right halves may show small defects in the cut sides. It is astonishing that the plutei in the experiment with fusion of halves from different eggs look more typical than many half larvae. It seems as if the bringing together of halves with a constitution varying as to the position of the ventral centre or parts of it, necessarily releases a very profound re-organization, leading to a new system. In such a system one ventral centre will be formed and become so strong that it dominates over the fragments of other centres that may have been present after the operation.

In the giant larvae two presumptive ventral centres are present after the fusion, either complete or split into parts (cf. Text-fig. 1 D H). Nevertheless, typical plutei may arise (Text-fig. 4). One strong centre became established, and it suppressed the other ones. The first detectable sign of another centre trying to assert itself is a supernumerary skeletal rod in an opposite meridian, the most dorsal part of the pluteus (Text-figs. 6, 7 A C). This gives the same picture as in a dorsal half isolated in the blastula stage where the dorso-ventral axis cannot be completely inverted (Hörstadius & Wolsky, 1936). Also an initial sign of irregularity is a supernumerary dorsal strip of ciliated band (Text-fig. 7D, cf. also 2D). The next step in the assertion of a second ventral centre is the appearance of both a dorsal rod and a dorsal ciliated band (Text-figs. 7 A–C, 8A). We then come to the double plutei with two oral fields and two pairs of skeletons (Text-figs. 8B, 10). In the oval gastrula the latter were formed at opposite ends. Only in two cases (Text-fig. 9) are the two partners in the same body more or less side by side, perhaps a result of two complete presumptive central centres of equal strength keeping in balance.

As indicated in Text-fig. 2 D–H, we must assume all sorts of combinations of two complete centres or parts of them, therefore often three or even four possible starting-points for ventral sides. In the light of this the actual series—giant plutei–plutei with dorsal rod–plutei with dorsal rod and band–double plutei—all without any anlage of a third or fourth skeleton or oral field, is rather remarkable and illustrates profound processes of reorganization.

SUMMARY

After exchange of meridional halves between two eggs typical plutei were obtained in nearly all pairs (Text-fig. 2).

Two 32-cell stages were split along a meridian, flattened, and fused (Text-fig. 3). There resulted typical giant plutei (Text-fig. 4); plutei with a supernumerary rod at the dorsal pole (Text-fig. 6); plutei with a supernumerary dorsal strip of ciliated band (Text-fig. 7D); plutei with both supernumerary rod and a ciliated band in the dorsal part (Text-figs. 7 A–C, 8A); joined twins (Text-fig. 9); and plutei with two ventral sides (Text-figs. 8B, 10).

The results are interpreted in the light of the hypothesis that there is a dominating presumptive ventral centre capable of subduing the tendencies toward formation of a ventral side which are located in other parts of the egg.

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(Manuscript received 7:v:56)

The Action of Testosterone and Oestradiol on the Sebaceous Glands and Epidermis of the Rat

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INTRODUCTION

THAT androgens increase the size of sebaceous glands is well known (de Graaf, 1943; Ebling, 1948; Montagna & Kenyon, 1949; Hamilton & Montagna, 1950; Haskin, Lasher, & Rothman, 1953). This effect might be due to increased cell proliferation, since it has been shown that testosterone stimulates mitosis in the epidermis of the mouse (Bullough & Van Oordt, 1950) or to increased cell size. But these are not the only possible explanations. Oestrogen has a marked effect in reducing epidermal thickness and the size of sebaceous glands without reducing the incidence of mitoses and it has been suggested that this is due to accelerated loss of cells (Hooker & Pfeiffer, 1943; Ebling, 1948, 1954, 1955). Thus testosterone might produce opposite effects by decreasing the rate of cell loss. Moreover, it must not be assumed that sex hormones have the same effect in the rat as in the mouse; e.g. the effect of oestrogen in stimulating epidermal mitosis in the mouse, reported by Bullough (1946, 1950 *a*, *b*), appears to be lacking in the adult rat (Carter, 1953; Ebling, 1954, 1955). For these reasons it seemed desirable to investigate the mechanism of the action of testosterone on the epidermis and sebaceous glands of the rat.

MATERIALS AND METHODS

Design of experiments and histological methods

Forty female Wistar rats from a small randomly mated colony were used for the experiments, which were designed so that the rats were killed when about 25 weeks old. All rats were spayed 19–22 days before death. On the day when they were killed at 1500 hours they were injected intraperitoneally at 1000 hours with 0·1 mg. colchicine in water per 100 g. body-weight. The use of colchicine for the determination of the incidence of epidermal mitoses in the rat has already been discussed (Ebling, 1954).

At the time of spaying the rats were divided into four groups of ten which were treated as follows:

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I. Untreated controls.
II. Implanted subcutaneously with about 8 mg. of testosterone.
III. Implanted with 8 mg. testosterone and about 5 mg. of 1:9 oestradiol/cholesterol.
IV. Implanted with 5 mg. of 1:9 oestradiol/cholesterol.

The implants were weighed after removal; the average absorption of testosterone appeared to have been roughly of the order of 0·3 mg./day, and of oestradiol 2–4 μg./day. These figures are in general agreement with amounts calculated from the known rates of absorption in man in relation to the surface area of the implants. The data of the manufacturers (Organon Laboratories) suggest that, on average, 0·2 mg. of testosterone and 3 μg. oestradiol would be absorbed each day.

The skins were removed and fixed as described in a previous paper (Ebling, 1954). Samples taken from near the mid-line in the anterior region of the back were embedded in ester wax (Steedman, 1947), sectioned sagittally at 7 μ, and stained in Ehrlich's haematoxylin and alcoholic eosin.

Whole mounts were prepared from similar pieces of skin about 1 cm. square by staining them for about 24 hours in a mixture of absolute alcohol, sodium hydroxide, and Sudan IV. Subcutaneous fat and the panniculus carnosus were dissected off, and the skin was mounted in glycerine jelly (Badertscher, 1940).

Measurements on sebaceous glands

The outlines of all the sebaceous glands in a length of skin section (15 mm.) were traced by means of a camera lucida and their areas determined by means of an 'Allbrit' precision disk planimeter. The number of nuclei visible in each gland-section was also recorded. These measurements will subsequently be referred to by the following symbols:

Total area of sebaceous glands in a section of 15 mm. length $=T$
Total number of gland sections (alveolar count) $=A$
Total number of nuclei (sebaceous cell count) $=S$

Calculation of mean alveolar volume

If a sphere is cut into thin slices of equal thickness its volume is equal to the product of the total area of the slices and their thickness.

Hence volume of sphere $\left(\frac{4}{3}\pi r^3\right)$

$$= \text{average area} \times \text{number of slices} \times \text{thickness.}$$
$$= \text{average area of slices} \times \text{diameter of sphere.}$$

Thus average area $= \frac{2}{3}\pi r^2$

and diameter $(2r) = 2\sqrt{\left(\frac{\text{average area} \times 3}{2\pi}\right)}.$

$$\begin{aligned}\text{Hence volume } &= 2\sqrt{\left(\frac{\text{average area}\times 3}{2\pi}\right)}\times\text{average area}\\ &= (\text{average area})^{\frac{3}{2}}\times 2\sqrt{(\tfrac{3}{2}\pi)}\\ &= (\text{average area})^{\frac{3}{2}}\times 1{\cdot}382.\end{aligned}$$

On the assumption that the sebaceous alveoli are spherical then the mean volume will be given by

$$\left(\frac{T}{A}\right)^{\frac{3}{2}}\times 1{\cdot}382.$$

Calculation of mean alveolar number

If the groups of glands are evenly distributed throughout the skin, then the total volume of sebaceous gland is equal to the product of the total area exposed on a cut face and the thickness of the block. By dividing this total volume by the mean volume it is possible to calculate the number of alveoli in the block. Thus number of alveoli per square millimetre of skin

$$\begin{aligned}&= \frac{\text{total gland area } (\mu^2) \text{ per millimetre of section}\times 1000}{(\text{average area})^{\frac{3}{2}}\ \times 1{\cdot}382}\\ &= \frac{\text{average area}\times\text{number of alveolar sections per millimetre}\times 1000}{(\text{average area})^{\frac{3}{2}}\ \times 1{\cdot}382}\\ &= \frac{\text{number of alveolar sections per millimetre}\times 1000}{\sqrt{(\text{average area})}\times 1{\cdot}382.}\end{aligned}$$

$$\text{Since average area} = \frac{T}{A}$$

$$\begin{aligned}\text{number of alveoli per square millimetre} &= \frac{A}{15\sqrt{\dfrac{T}{A}}}\times\frac{1000}{1{\cdot}382}\\ &= \sqrt{\left(\frac{A^3}{T}\right)}\times 48{\cdot}24.\end{aligned}$$

Estimation of alveolar number from whole mounts

The number of alveoli per square millimetre was also estimated in whole mounts of skin from counts of ten fields (2·01 mm. in diameter) for each rat.

Calculation of cell volume

The cell volume is clearly given by

$$\frac{T\times\text{section thickness}}{S}.$$

The sebaceous cell count could be erroneously high owing to fragments being counted as whole nuclei, and it is possible to correct for this by methods due to Abercrombie (1946) if absolute estimates are required. However, in the present work it was considered sufficient to neglect fragments which were clearly less than half nuclei, that is to say those which had an optical section of less than normal size.

Epidermal thickness, incidence of mitoses in sebaceous glands, and incidence of mitoses in epidermis

The thickness of the stratum germinativum plus stratum granulosum or epidermal thickness (measured from base of stratum germinativum to base of stratum corneum), the number of arrested metaphases per fifty sebaceous gland sections, and the number of metaphases per 1 cm. length of stratum germinativum, were all determined as previously described (Ebling, 1954).

RESULTS

Sebaceous glands

In Table 1 the actual observations are summarized; the transformed figures shown in Tables 2 and 4 are calculated not from the means shown in Table 1 but from the individual results for each rat.

TABLE 1

Effect of testosterone, oestradiol, and testosterone plus oestradiol on sebaceous glands of spayed rats aged 25 weeks (results expressed as means ± S.E.)

Group	*Treatment*	*Total area of gland sections in 15 mm. (T) sq. μ*	*Number of gland sections in 15 mm. (alveolar count = A)*	*Number of sebaceous cells in 15 mm. (sebaceous cell count = S)*	*Mitoses per fifty gland sections in 5 hours*
I	Controls	79,781±4,760	27·7±1·7	286·4±16·8	14·6±1·3
II	Testosterone	131,484±8,463*	30·4±1·4 NS	408·3±25·1*	26·6±1·4*
III	Testosterone and oestradiol	105,467±9,801†	30·9±2·0 NS	331·5±29·7 NS	25·8±4·2†
IV	Oestradiol	41,423±6,114*	20·9±1·2‡	153·7±18·7*	14·2±1·6 NS

Comparison with controls: NS = not significantly different.

* $P < 0{\cdot}001$. † $P = 0{\cdot}05 - 0{\cdot}02$. ‡ $P = 0{\cdot}02 - 0{\cdot}01$. § $P = 0{\cdot}01 - 0{\cdot}001$.

It is clear (Table 2) that alveolar volume was very significantly increased, on average by about 80 per cent., in the rats which were implanted with testosterone, whereas oestradiol reduced the mean volume by about one-half. In no groups were there any significant changes in the alveolar number as calculated from sagittal sections. Since the glands tend to be sac-shaped rather than spherical, an exaggeration of alveolar volume with a corresponding underestimate of alveolar number might have been expected. An examination of the alveolar numbers as estimated from whole mounts (Table 3) shows that such an error is very slight and significant only in the testosterone-treated group in which the glands were very large. This suggests that the estimates of alveolar volume in Table 2 are reasonably accurate. In Table 3 alveolar number appears to have been slightly higher in rats treated with both testosterone and oestradiol than in the other groups. However, this may well be because the glands of the rats treated with testosterone stained much more satisfactorily than the others.

Table 2

Sebaceous alveolar volume, alveolar number, and cell volume as calculated from sagittal sections of skin (results expressed as means ± S.E.)

Group	*Treatment*	*Alveolar volume (cu. μ)*	*Alveolar number per sq. mm.*	*Cell volume (cu. μ)*
I	Controls	222,070±20,274	25·25±2·18	1955±47
II	Testosterone	400,923±29,530*	22·51±1·19 NS	2276±108‡
III	Testosterone and oestradiol	271,776±18,296 NS	25·70±1·39 NS	2220±80‡
IV	Oestradiol	117,908±14,702*	23·08±0·72 NS	1858±64 NS

For explanation of symbols see Table 1.

Table 3

Sebaceous alveolar number as estimated from whole mounts of skin

Group	*Treatment*	*Alveolar number per sq. mm. (means±S.E.)*	*Range*
I	Controls	25·04±0·56	22·0–27·3
II	Testosterone	26·75±1·07	21·4–32·4
III	Testosterone and oestradiol	27·83±0·62§	24·8–30·7
IV	Oestradiol	24·07±0·74	20·3–28·4

For explanation of symbols see Table 1.

The effect of testosterone on alveolar volume is in part due to a significant increase (about 16 per cent. on average) in cell volume (Table 2). By dividing cell volume into alveolar volume an estimate of the number of cells per gland may be made. This shows that testosterone caused a very significant increase in cell number, amounting to about 60 per cent. (Table 4). The question of whether this is due to increased cell proliferation or decreased cell loss now arises. It appears from Table 1 that the incidence of mitoses per fifty gland sections per 5 hours was significantly increased. The total number of cells visible in fifty gland sections can be calculated from the sebaceous cell and alveolar counts and thus it is possible to estimate the proportion of cells undergoing mitosis in 5 hours and from this to make a comparative estimate of the number of cells produced per gland per day (Table 4). As these figures make the unwarranted assumption that the incidence of cell division does not vary diurnally, and as no absolute value can be assigned to mitotic counts made with the use of colchicine, they must be regarded of value only for comparative purposes. If the total number of cells per gland is divided by the number produced per day it is possible to make an estimate of the time of cell survival, shown in Table 4 as the 'estimated cell life'. The results show that testosterone more than doubled the mean rate of cell proliferation in the glands, but elicit the interesting fact that at the same time the length of cell life was decreased, i.e. the rate of cell loss was increased.

TABLE 4

Cell proliferation and cell life in sebaceous glands (results expressed as means ± S.E.)

Group	*Treatment*	*Number of cells in gland*	*Number of new cells per gland per day*	*Estimated cell life in days*
I	Controls	112·5±8·7	14·9±1·5	8·1±0·82
II	Testosterone	176·4±10·8*	35·2±2·1*	5·1±0·23§
III	Testosterone and oestradiol	122·2±7·1 NS	28·1±4·4‡	5·4±0·92†
IV	Oestradiol	62·8±6·4*	11·4±1·2 NS	6·3±0·99 NS

For explanation of symbols see Table 1.

The analysis of the mode of action of oestradiol is not quite so clear cut. Cell volume was not significantly affected (Table 2). The number of cells per gland was approximately halved (Table 4), although the number of mitoses per fifty gland sections was not affected (Table 1). The possibility that cell proliferation was slightly reduced cannot, however, be ruled out (Table 4), and it seems likely that the cell life was lowered, although the difference in the means is not statistically significant.

In the rats treated with both testosterone and oestradiol the mean volume of the sebaceous glands was only about 20 per cent. greater than in the controls, and the difference is not statistically significant. Moreover, the number of cells per gland is not significantly above normal (Table 4). Nevertheless, it appears that each hormone retained its individual effects. The cell volume was equal to that of rats treated with testosterone alone and significantly higher than that of the controls. The rate of cell proliferation was double that of untreated rats, and the estimated cell life significantly less.

Epidermis

The results are shown in Table 5. Testosterone did not affect epidermal thickness, nor was the incidence of mitoses significantly changed. It is not impossible, however, that testosterone produced a slight increase in the incidence of mitoses in both groups II and III. Oestradiol caused a significant decrease in epidermal thickness which was clearly not due to decreased cell proliferation; therefore it seems that cell loss must have been accelerated. A decrease in epidermal thickness probably occurred in the rats treated with both hormones, although the difference in the means is not statistically significant.

TABLE 5

Effect of testosterone, oestradiol, and testosterone plus oestradiol on epidermis of spayed rats aged 25 weeks (results expressed as means ± S.E.)

Group	*Treatment*	*Thickness of stratum germinativum plus stratum granulosum (μ)*	*Number of metaphases per 1 cm. length*
I	Controls	16·6±1·2	21·5±3·2
II	Testosterone	16·2±0·8 NS	27·6±6·4 NS
III	Testosterone and oestradiol	14·1±0·9 NS	30·7±4·7 NS
IV	Oestradiol	13·5±0·8†	22·2±4·6 NS

For explanation of symbols see Table 1.

DISCUSSION

From the present study it appears that the enlargement of the sebaceous glands, induced in the rat by testosterone, is caused partly by an increase in cell volume, which accounts for about one-quarter of the effect, but mainly by an increase in cell proliferation. At the same time the rate of breakdown of the cells is accelerated, that is to say their length of life is reduced. These effects on cell size and cell proliferation seem to retain their identity even when oestradiol, which by itself reduces the size of the glands, is given at the same time as testosterone, even though such glands may not be enlarged.

The mechanism by which oestradiol reduces gland size is not quite so clearly demonstrated. It does not affect cell size in adult spayed rats. Although in these experiments the reduction in mean cell life was not statistically significant, there seems to be little doubt that oestradiol does accelerate cell breakdown in both sebaceous glands and epidermis. It has, for example, been shown that in immature rats these structures can be greatly reduced in size at the same time as the incidence of mitoses is increased (Ebling, 1954) and other evidence in support of such an action has already been presented (Ebling, 1955). But it does seem possible that a decrease in cell proliferation may account for a minor part of the reduction in gland size in the present study.

Lapière (1953) has studied the effect of testosterone and oestradiol applied in oil to the skin of mice. The present observations on the rat are in keeping with his conclusion that testosterone increased the diameter of the sebaceous glands by increasing both cell size and mitotic index. He claims, however, that the mode of action of oestradiol is exactly opposite to that of testosterone, i.e. that it reduces both cell volume and the mitotic index. My evidence does not support either of these conclusions. Cell volume was not significantly altered, and it seems unlikely that a lowered incidence of mitoses could explain more than a small part of the effect of oestradiol on the sebaceous glands of the rat. Moreover, even in the mouse such a conclusion may not be a correct inference from Lapière's data. The 'mitotic index', which is estimated without the use of colchicine, is the number of cells per 1,000 that are undergoing mitosis at the instant of fixation of the tissue. This index depends, therefore, not only on the number of new cells being produced but also on the duration of mitosis. Thus a lowered mitotic index could be due to a speeding up of mitosis, so that there were fewer cells in division at any instant, and need not indicate a lower production of cells. Bullough (1950*b*) has presented evidence that oestrogens do increase the speed of mitosis in this way. Finally, even if it were true that oestradiol did not alter the duration of mitosis, it would then follow that a reduced mitotic index must indicate a reduced rate of cell loss as well as reduced cell proliferation. It has been shown that such an effect is unlikely.

The results may throw some light on the suggestion that the human complaint *acne vulgaris*, which involves enlarged sebaceous glands, is due to an increase in the androgen/oestrogen ratio as put forward, for example, by Lawrence & Werthessen (1940) and Aron-Brunetière (1953). It would appear that although the size of the sebaceous glands depends on the androgen/oestrogen ratio, the amount of sebum actually secreted, which is dependent only on cell proliferation and cell volume, is affected mainly by the amount of androgen. A rough comparative estimate of glandular output in the experiments can be made from the data in Tables 2 and 4. Testosterone increased the mean output from about 29,000 cu. μ to over 80,000 cu. μ per gland per day. Oestradiol caused a slight reduction to about 21,000 cu. μ. In rats treated with both hormones the mean output was 62,000 cu. μ. These figures emphasize that although superficially oestradiol and testosterone appear to affect gland size in opposite ways, their individual effects are in fact maintained when both hormones are administered at the same time.

SUMMARY

1. A method is described for estimating the mean volume and mean number of sebaceous glands per unit area from sections of skin.

2. The enlargement of the sebaceous glands which resulted from the implantation of testosterone in spayed rats aged 25 weeks was shown to be due to increases in both cell proliferation and cell volume; at the same time cell loss appeared to have been accelerated.

3. The effect of oestradiol, which reduced the volume of the sebaceous glands, appeared to be due mainly to an acceleration of cell breakdown rather than to an inhibition of cell proliferation; cell size was not affected.

4. When both hormones were administered simultaneously the identity of their individual effects appeared to be maintained, although the size of the resulting glands was not significantly different from normal.

5. Oestradiol reduced the thickness of the epidermis (stratum germinativum plus stratum granulosum) in spayed rats without altering the incidence of mitoses, but testosterone had no significant effect.

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The Effect of Maternal Immunization against Organ Tissues on Embryonic Differentiation in the Mouse

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WITH TWO PLATES

INTRODUCTION

THE role of mechanisms resembling those of antibody formation in processes of embryonic differentiation has been discussed widely in recent years. Such considerations have led, for example, to the development of the auto-antibody concept by Tyler who states 'that each of the various macromolecular substances of which cells are constructed bears the same sort of relationship to another of these substances as do antigen and antibody and that their mode of origin is analogous to that of antibody formation'. The applicability of this concept to problems of differentiation and even gene action has been discussed in some detail recently (Tyler, 1955).

In search for a mechanism by which certain mutations in the house mouse with severe effects on early embryonic differentiation (for review cf. Gluecksohn-Waelsch, 1953) might produce their effects, the possibility presented itself that these particular mutations might interfere with normal differentiation by way of immune type processes. Before an attempt could be made to attack this problem in the mutants, it was necessary to find out how the developing organ systems of normal mouse embryos might react to the action of antibodies. For this purpose normal adult female mice were injected with emulsions of adult brain-tissue, and the possible effect of the immunization against brain on the developing nervous system of subsequently conceived embryos was investigated. The results of these experiments are reported and discussed in this communication. Preliminary reports of these findings have been published (Gluecksohn-Waelsch, 1954, 1955 *a*, *b*).

MATERIALS AND METHODS

The following normal strains of mice were used in the experiments:

Balb/C Glw (F90);

DBA/1 Glw (obtained from the R. B. Jackson Memorial Laboratory and

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subsequently inbred in our laboratory by brother–sister mating for 3–4 generations);

DBA/1 Jax;

Swiss Webster albino, not inbred (as donors of brain-tissue only).

Antigenic emulsions for the immunization of female mice were prepared with brain-tissue and with heart-tissue. The brain-emulsions were made up in the following way:

For each batch of emulsion the brains were dissected from 50 normal adult mice under sterile conditions and frozen in the deep freeze immediately. Subsequently the organs were suspended in phenolized saline and disintegrated in a Waring blendor; the emulsion was combined with adjuvants following Freund's technique (1942). The following proportions were used: 10 g. brain, 14 c.c. phenolized saline, 28 c.c. paraffin oil, 14 c.c. aquaphor, 35 mg. killed tubercle bacilli. The final emulsion thus contained 15 per cent. brain tissue.

The heart-emulsions were prepared in the same way from 50 mice for each batch and combined with Freund's adjuvants in the same proportions; these emulsions contained 15 per cent. heart-tissue.

Sites of injection were hind legs predominantly; occasionally intra-abdominal injections were given as recorded in the experiments described below. Dosages were determined in such a way as to stay below the dose which might induce experimental encephalomyelitis (Morgan, 1947) and thus interfere with the breeding of treated females. Usually—unless noted otherwise—0·2 c.c. of the emulsion were injected at one time, 0·1 c.c. into each leg. Three injections of 0·2 c.c. were given at intervals of 1 week, so that each animal received three injections within 2 weeks. After the third injection the injected females were paired with normal males of their own strain and were checked twice daily for copulation plugs. The majority of females mated normally. Pregnant females were dissected and the embryos removed for study, preferably at 10 days after the observation of the copulation plugs. This particular day was chosen because at this stage abnormalities of the nervous system can be easily recognized; severely affected embryos, on the other hand, would not have died yet and been resorbed. Before dissection heart-blood was obtained from the pregnant females and serum was prepared for possible serological studies.

RESULTS

In the first series of experiments mice of the Bagg albino strain were used as recipients of the emulsion, which had been prepared with brain from Swiss Webster males. This Bagg albino strain had been chosen because it had previously been reported to be free of sporadic abnormalities of the nervous system.

Table 1 shows the results of this experiment: of 41 females injected with the antigenic emulsion and allowed to mate, 37 became pregnant and 321 embryos were obtained from these mothers. Twenty of these embryos showed abnormalities of the nervous system and 16 were dead and resorbed. Surprisingly enough, however, abnormalities were found also in the uninjected controls, though in lower frequency. Thus, this Balb strain, upon closer inspection, proved actually not to be free of sporadic abnormalities of the nervous system. A great deal of interest attaches, of course, to the finding of the increase of abnormalities over the sporadic incidence under the conditions of this experiment, particularly in view of Goldschmidt's (1956) recent discussion of the theoretical basis of such susceptibility; nevertheless, it seemed of questionable value to continue a study of the developmental effects of antibodies with a strain which reacts to many different sorts of agents with the formation of nervous system abnormalities. The possible specificity of such an effect cannot be studied in a generally susceptible strain. In the remaining experiments DBA/1Glw mice were therefore used as recipients of the antigenic emulsions; this strain was derived from the DBA/1 Jax strain three generations before.

TABLE 1

Summary of results obtained with the Balb/Glw strain of mice

Experiment	*No. of females injected*	*No. pregnant*	*No. of embryos*	*Normal*	*Abnormal nervous system*	*Miscellaneous abnormalities*	*Dead and resorbed*
1	41	37	321	285	20	0	16
2	uninjected controls	37	281	261	11	0	9

TABLE 2

Summary of results obtained with DBA/1 strain

Experiment	*Type of emulsion and no. of females injected*	*No. pregnant*	*No. of embryos*	*Normal*	*Abnormal nervous system Actual*	*%*	*Miscellaneous abnormalities*	*Dead and resorbed*
3	Brain: 20	17	147	114	12	9·3	0	21
4*a*	Heart: 28 intramuscular	12	105	90	0	0	4	11
4*b*	Heart: 12 intra-abdominal	5	29	22	0	0	1	6
5	Brain: 17	14	109	92	8	7·9	1	8
6	Heart: 12	10	80	73	0	0	1	6
All Brain: 37		31	256	207	20	8·8	1	28
All Heart: 52		27	214	185	0	0	6	23

The DBA/1 mice were treated as described above: they received three injections of 0·2 c.c. of the brain emulsion at weekly intervals and were mated subsequently. Sibs of these brain-injected animals were injected in the same manner with a heart-emulsion and served as controls. In experiment 3 brain from the Swiss Webster strain and in experiments 4 *a* and *b*, 5, and 6, brain or heart from the DBA/1 Jax strain were used in making up the antigenic emulsions. Table 2 shows the results of these experiments.

It appears from these figures that abnormalities of the nervous system are found exclusively in the embryos from mothers injected with the brain-emulsion. The incidence of nervous-system abnormalities is consistent in the two brain series, i.e. about 8 to 9 per cent. of all observable embryos show them. The group under the heading 'miscellaneous abnormalities' contains one embryo with an abnormal heart in experiment 4*b*; other abnormalities in experiments 4*a* and 6 consist in complete suppression of embryonic differentiation in three cases and suppression of posterior trunk development in the remaining two. In the brain series only one miscellaneous abnormality appears in experiment 5 where differentiation of embryonic structures is suppressed completely.

Description of nervous-system abnormalities

The abnormalities of the nervous system observed in the embryos of brain-injected mothers vary from suppression of nervous-tissue differentiation in the region of the brain and the anterior spinal cord to microcephaly and abnormalities of closure of neural folds. Fig. A of Plate 1 is a photograph of an embryo dissected from its mother at 10 days after copulation; this mother had been injected with brain-emulsion in experiment 5. Gross observation reveals absence or extreme reduction of the brain folds and anterior neural folds; head and dorsal anterior region appear as a transparent hollow vesicle covered by thin epidermis; the ear vesicle is clearly visible. This embryo was alive at dissection; its heart beat strongly, and somites and well-formed trunk and tail structures were observed. A cross-section through the ear vesicle region of the abnormal embryo (Plate 1, fig. B) reveals the thinness of the neural tube walls in contrast to the normal condition illustrated in fig. C of Plate 1. Another abnormal embryo with deficiency of neural tissue formation obtained in experiment 3 is seen in Plate 1, fig. D. Here also the nervous system appears very abnormal: there is some tissue in the forebrain region but hardly any in the rest of the head and the anterior neural tube regions; a hollow vesicle covered by thin epidermis, collapsed in the specimen illustrated here, with clearly outstanding ear vesicles forms the anterior part of this embryo. Mesodermal structures, e.g. somites, appear well developed in the posterior part of the body. The embryo was alive at dissection and its heart beat well. A cross-section through the ear vesicle region is seen in Plate 1, fig. E, and demonstrates the thinness of the neural tube wall in contrast to the normal condition in fig. C. Further abnormalities are illustrated in the subsequent figures. Fig. F of Plate 2 shows a normal and an abnormal embryo dissected from the mother at 10 days after copulation. The mother had been injected with the brain-emulsion in experiment 5. The brain folds of the abnormal embryo appear as large empty vesicles, six pairs of somites are counted, and trunk and tail formation seem normal. Open-head folds at a stage when they would have closed normally are shown in an abnormal embryo in Plate 2, fig. G; it is 10 days old and from a brain-injected mother. A normal litter-mate is shown for comparison on the same picture. An example of microcephaly is illustrated in Plate 2, fig. H, where the embryo in the centre is normal and the two on either side are abnormal.

DISCUSSION

The experiments reported here demonstrate that 8–9 per cent. of all embryos from mothers injected with an emulsion of brain and Freund's adjuvants show developmental abnormalities of the nervous system, whereas the embryos of uninjected controls or of females treated with a heart-emulsion have normal nervous systems.

A number of questions arise in the interpretation of these results. First of all, one would like to know which of the ingredients of the injected emulsion is responsible for the effect. A partial answer to this question can be obtained from the control experiments. Whereas 20 out of 228 observable embryos from brain-injected mothers show abnormalities of the developing nervous system, not a single neural abnormality is recorded among the 191 observable embryos from heart-injected mothers.

These results seem to indicate strongly that the adjuvant ingredients of the antigenic emulsions do not have any effect on development and are not responsible for the abnormalities observed since the adjuvants were identical in brain-injected and heart-injected mothers. It appears, furthermore, unlikely that the adjuvants could have exerted any direct deleterious effect on development because the injections were given before the animals were permitted to mate. The actual time of copulation varied in the different experimental animals and fell between 4 days and 9 weeks after the third and last injection.

An unspecific antigenic effect of adult mouse tissue does not seem to be responsible for the developmental abnormalities, either, in view of the failure of embryonic abnormalities of the central nervous system to appear after heart injections in contrast to the results with brain injections. Thus, the choice of ingredients of the antigenic emulsion possibly responsible for the abnormalities observed is narrowed down to brain-tissue. In discussing mechanisms by which the injection of brain-tissue might produce its effect, one has to take into account the possibility that certain brain constituents might persist in the injected mouse and be transmitted across the maternal-fetal barriers to the embryo to exert their effect on differentiation directly. There exists no evidence at present which would exclude this possibility. On the one hand, such retention of active ingredients seems unlikely on the basis of considerable evidence in favour of rapid disintegration of most types of antigenic molecules in the host organism cited by Burnet & Fenner (1953). On the other hand, it is significant in this connexion to remember that the work of Olitsky & Lee (1953) points towards a proteolipide fraction as the encephalitogenic agent in the injection of central nervous system tissue into mice; it is not known whether such proteolipides may not be preserved in the organism for a considerable time after injection. Further work would be required, of course, to show if this same proteolipide fraction is the active agent in the experiments reported here but in the consideration of the present results the possibility must be kept in mind of a direct effect of a constituent of the injected brain-tissue retained in the maternal organism and transmitted to the embryo.

In spite of these reservations, it appears more likely that the abnormalities of the nervous system observed in the present experiments are due to a direct effect of maternal antibodies against brain on the developing nervous system of the embryos. It is, however, not possible on the basis of the present experiments to ascribe the results reported here to antibodies specific for brain-tissue only. Great caution must be observed in attributing results obtained with crude brain-tissue to antibodies specific for brain; organ-specific as well as non-specific antibodies were demonstrated, for example, in sera of monkeys injected with brain-tissue or brain-extracts (cf. Lumsden *et al*., 1950). Nevertheless, the fact remains in the experiments and data presented here, of the specific relationship between injection of brain-tissue and reaction of differentiating nervous system: the nervous system appeared to be the only part of the embryo to react to administration of brain-tissue to the mother, and brain-emulsion only was able to evoke this response in the embryo under the experimental conditions described here.

Some preliminary serological results obtained with the sera of immunized mothers might be of interest in this connexion: through the kindness of Dr. B. Seegal at the College of Physicians and Surgeons, New York, 10 sera from brain-injected mothers with abnormal embryos were studied for their precipitin reaction to brain. These tests revealed a positive precipitin reaction in 7 sera from mothers with 1–3 abnormal embryos each. Three sera of mothers with only 1 abnormal embryo each failed to give a positive precipitin reaction. These preliminary results, while certainly not conclusive, are encouraging in that they indicate the presence of antibodies in the serum of brain-injected mothers with abnormal embryos.

A positive correlation between immunization of mothers against brain-tissue, on the one hand, and appearance of nervous-system abnormalities in the embryos of such mothers, on the other hand, thus seems to exist in our experimental material. This brings up the question whether maternal antibodies against brain are really able to reach the site of the developing embryo and by what route they might do so. The actual transmission of maternal antibodies to the mouse embryo *in utero* was demonstrated by Paul Ehrlich (1892) in studies of immunization of mice against toxic plant proteins, e.g. Ricin, Abrin, Robin. The route of transmission of antibodies from mother to foetus has been studied by Brambell (1954) in the rabbit in a series of beautiful experiments. The placenta itself was found to be impermeable to maternal antibodies, but instead, antibodies were found to pass from the maternal circulation into the uterine cavity from where they were absorbed by the embryonic membranes and thus transmitted to the foetus. In view of the fundamental similarities of placentation in the rabbit and the mouse it seems possible that the route of transmission of maternal antibodies may be similar in the two species, and that in our experiments with mice these antibodies are absorbed by the embryo through its membranes.

If maternal antibodies against brain-tissue actually traverse the barriers between mother and embryo and affect the developing nervous system, one would like at least to speculate about possible mechanisms by which such an effect may be produced. There exist several reports in the literature of experimental results bearing close relation to those reported here. The claims of Guyer & Smith (1924) to have produced heritable lens abnormalities in the offspring of rabbits injected with anti-lens sera, could not be confirmed by other authors, and have thus been an object of controversy for some time. The work of Ebert (1950), on the other hand, demonstrates without any doubt specific effects of anti-heart and anti-spleen sera on the differentiation of primarily mesodermal elements of the chick blastoderm, and there also seem to be specific effects of anti-brain sera on nervous-tissue differentiation. Into this same category of results belong the experiments of Freund *et al.* (1953) who were able to interfere with normal spermatogenesis in guinea-pigs by injection of an emulsion containing guinea-pig testis, or spermia, and adjuvants. The effect was specific and not the result of an inflammatory process.

In all these different experiments the results may be interpreted as being due to an interference of antibodies to specific tissues with the differentiation of the corresponding organ systems: heart, spleen, and nervous-tissue differentiation were affected by anti-heart, anti-spleen, and anti-brain sera respectively in Ebert's experiments on chicks, the differentiation of spermatozoa from spermatocytes was inhibited in Freund's experiments by antibodies to testis or sperm, and antibodies to brain seemed to affect specifically the differentiation of nervous tissue in the experiments reported in this paper. All these results appear to be of interest in view of the hypothesis that processes of immunological nature resembling antigen-antibody type of reactions may be operative in embryonic differentiation and that it might be possible, in turn, to interfere with these processes by immunological means. In the present experiments such interference seems to have been achieved by the experimental production of antibodies in the maternal organism. One might visualize such an interference as the result of gene action in the case of certain mutations, and it is planned to extend the experimental work reported here towards such a possibility, specifically in connexion with some of the Brachyury mutants in the house mouse which show severe effects on early embryonic growth and differentiation (cf. Gluecksohn-Waelsch, 1953).

In the experiments reported here the incidence of nervous-system abnormalities is about 8–9 per cent. of all observable embryos. This particular incidence is without doubt the result of a number of different factors: first of all it is well known that individual animals, even of a highly inbred strain, vary in the intensity of their immune response, so that individual female mice injected with brain-emulsion might have produced different amounts of antibodies. Support for this argument may be found in the fact that the 8–9 per cent. (actually 20) abnormalities observed were distributed among 11 litters whereas the remaining 20 litters were completely unaffected. Furthermore, there probably is considerable variation in the transmission of maternal antibodies to the foetus in different individuals. Finally, the susceptibility or resistance of the individual embryos themselves introduces still another variable. One would expect all these variables to differ in different inbred strains of mice so that the total incidence of nervous-system abnormalities would vary accordingly in different strains exposed to the identical treatment.

The practically complete absence of heart abnormalities in the experiments with heart-injected mothers (1 abnormal heart in 214 embryos, cf. Table 2) deserves some comment. There is, in general, an amazing paucity of reports of heart abnormalities in the mouse in response to traumata of exogenous or endogenous nature. This innate resistance to injuries of the heart-forming material in the mouse may account also for the present results. The apparently high ability of regulation of the developing heart has been discussed before (Gluecksohn-Waelsch, 1955*a*).

One might also try to interpret the absence of heart abnormalities in these experiments in the light of recent work on tolerance (Billingham *et al.*, 1956): exposure of the future antibody-forming system of the developing mouse to the effect of heart-tissue antigenic substances carried through the blood-stream might result in acquired tolerance to these antigens in the adult organism. Consequently, one might expect a weaker immune response of the mother to the injection of heart-tissue than to that of, for example, brain-tissue, which during development is relatively more isolated from the antibody-forming system.

SUMMARY

1. The response of embryonic differentiation to injection of organ-tissue-emulsion into female mice prior to conception was studied.

2. The injection of brain-emulsion into females of the DBA/1 strain was followed by abnormalities of the developing nervous system in 8–9 per cent. of subsequently conceived embryos. Such abnormalities consisted of suppression of nervous-tissue differentiation, microcephaly, and abnormalities of closure of neural folds.

3. As controls, female sibs of brain-injected mothers were injected with a heart-emulsion. No nervous-system abnormalities were observed in the subsequently conceived embryos of these mothers.

4. The possible mechanism of the effect described here is discussed. It seems most likely that maternal antibodies are responsible for the nervous-system abnormalities of the embryos. Problems of the transmission of these antibodies

to the embryo and of the mechanism of their effect on embryonic differentiation are discussed.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES

Plate 1

Fig. A. Photograph of mouse embryo 10½ days after fertilization, from mother injected with brain-emulsion. Note suppression of nervous-tissue differentiation. *E.V.*, ear vesicle; *so.*, somites. × 18.

Fig. B. Cross-section through ear vesicle region of same abnormal embryo. *B.V.*, brain vesicle; *B.W.*, brain wall. × 138.

Fig. C. Cross-section through ear vesicle region of normal embryo. × 63.

Fig. D. Photograph of mouse embryo 10½ days after fertilization, from mother injected with brain-emulsion. Note deficiency of nervous-tissue differentiation. *Abs. of n.t.*, absence of nervous tissue; *H.*, head; *h.*, heart. × 30.

Fig. E. Cross-section through ear vesicle region of same abnormal embryo. × 129.

Plate 2

Fig. F. Normal (right) and abnormal (left) mouse embryo 10 days after fertilization, from mother injected with brain-emulsion. *B.V.*, abnormal brain vesicles. × 11.

Fig. G. Normal (right) and abnormal (left) mouse embryo 10 days after fertilization, from mother injected with brain-emulsion. *O.H.F.*, open head folds. × 9.

Fig. H. Dorsal view of normal (centre) and abnormal (left and right) mouse embryo litter-mates 10 days after fertilization, from mother injected with brain-emulsion. *Microc.*, microcephalic. × 9.

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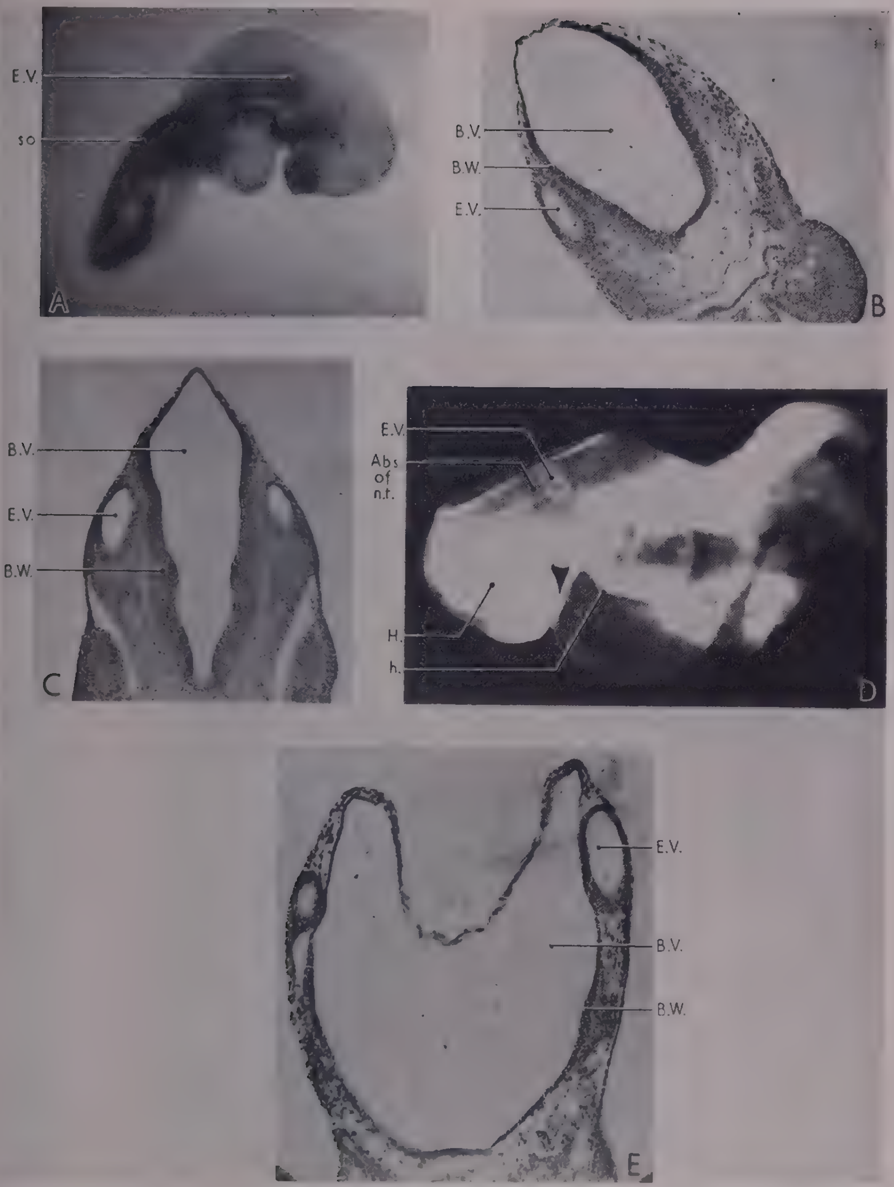
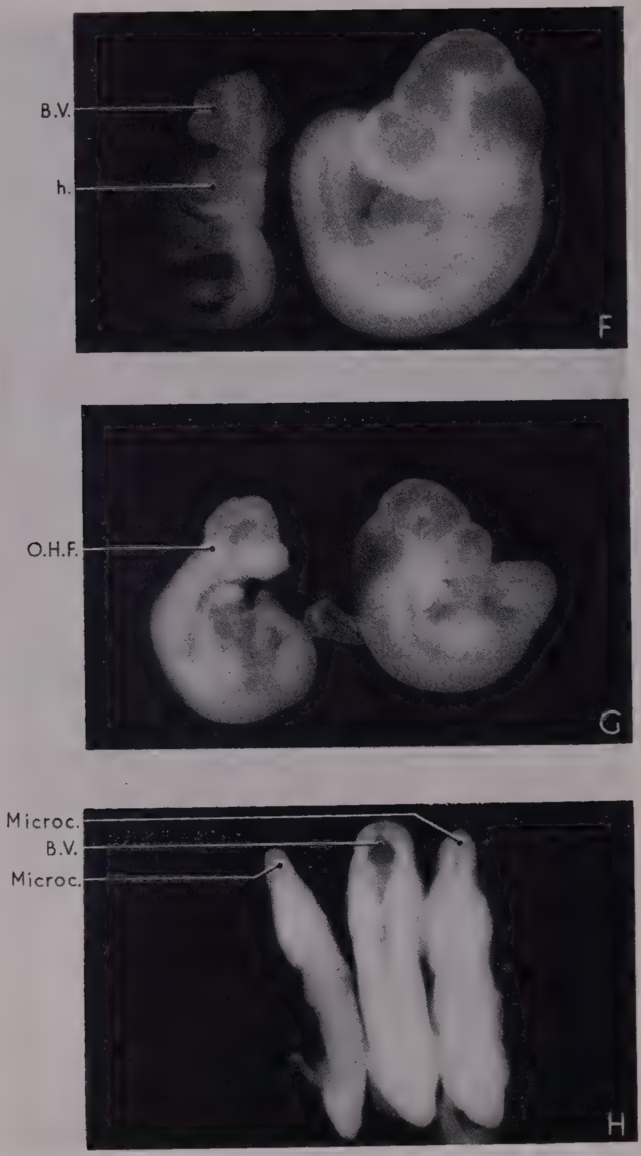


S. GLUECKSOHN-WAELSCH

Plate 1



S. GLUECKSOHN-WAELSCH

Plate 2

'Matted', a New Hair-mutant in the House-mouse: Genetics and Morphology

by A. G. SEARLE *and* R. I. SPEARMAN[1]

From the Medical Research Council Group for Experimental Research in Inherited Diseases, University College London

WITH ONE PLATE

THREE types of hereditary hairlessness are known in the house-mouse. With Naked (Fraser, 1946) and Alopecia (Dickie, 1955), baldness results from the breaking of hairs; with hairless and its allele rhino, from the shedding of entire hairs (Fraser, 1946). The crinkled (Falconer, Fraser, & King, 1951) and ragged (Carter & Phillips, 1954) genes, however, lead to a complete absence of certain hair-types, due to delayed or imperfect follicle development (agenesis). In rough (Falconer & Snell, 1952) the fur is greasy and looks unkempt but no baldness occurs.

Matted, the new mutant to be described in this paper, belongs to the first of these three categories. The degree of baldness varies greatly; there may be no signs of any hair loss, or a complete absence of fur over the back and flanks. The head and belly are not affected as a rule. The more extreme manifestation is associated particularly with the later stages of each coat generation. Whatever coat is present it looks and feels rough, in sharp contrast to the sleekness of the normal mouse. Instead of lying down flat against the body, the mutant's hairs tend to stand up as if inflexible and stick together at the tips so as to form matted clumps; a very characteristic feature (Plate, figs. A, B. C). In agouti mice this erection of the hair causes a darkening of coat colour, for the black basal part of each agouti hair becomes visible.

The present paper deals with the genetics of the new mutant and explains its effect in terms of the underlying abnormalities of hair structure. A paper by Jarrett & Spearman (1957) deals with finer structure and the results of chemical and physical studies.

GENETICS

The matted gene first appeared as a spontaneous mutation in the inbred CBA/Gr strain in 1952. The whole CBA stock was breeding poorly and looking out of condition at the time, but some mice were particularly unkempt, with

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bald patches on the back. These mice were outcrossed to the C57Bl/Gr strain. All F_1 offspring had normal sleek fur, but about one-quarter of the F_2 were of the matted phenotype (Table 1). Reciprocal backcrosses both gave ratios of matted to normal which did not differ significantly from 1 : 1, while matted × matted crosses gave only matted offspring. Thus it is clear that this hair abnormality is due to a single fully penetrant recessive autosomal gene for which the symbol *ma* is proposed. There is no evidence of any reduction in viability and fertility is high, many matted mothers having as many as ten litters, while the size of litters is normal. No linkage studies or tests for allelomorphism with other hair genes have yet been made.

TABLE 1

Segregation data for matted

Father	*Mother*	*Normal*	*Matted*	*Total*	*Percentage*
ma/*ma*	+/+	20	0	20	0
+/+	*ma*/*ma*				
+/*ma*	+/*ma*	249	80	329	24·3
ma/*ma*	+/*ma*	103	91	194	46·9
+/*ma*	*ma*/*ma*	154	132	286	46·2
ma/*ma*	*ma*/*ma*	0	107	107	100

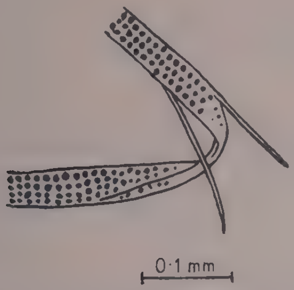
The age at which this mutant can first be identified on its external appearance varies between 2 and 4 weeks, being generally somewhat earlier in agouti than in black mice mainly owing to the former's abnormally dark appearance. Matted mice may appear almost normal with the growth of a new hair generation, but as the hair lengthens the abnormal aspect is regained. If the fur is stroked in the wrong direction the characteristic matted appearance of the hair is more easily seen.

MORPHOLOGY

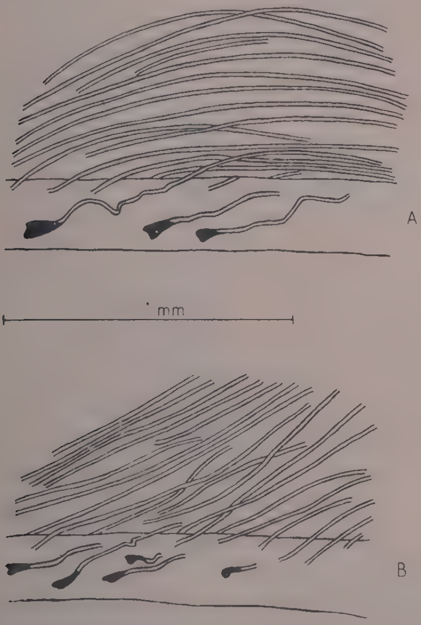
The external appearance of matted mice differs from normal in showing, (i) erection of hair, (ii) matting of hair in clumps, (iii) a tendency to baldness, (iv) a change to a brown colour of black melanin in old hairs. The explanation of the apparent paradox between this and the darkening of the agouti coat is that the proximal parts of agouti hairs even when changed in colour are darker than the tips.

Erection of hair

Hair erection might be caused by inflexibility, or by the angle between epidermis and emerging hair being greater than normal. Flexibility was tested by repeatedly bending a small number of hairs over the edge of a glass slide and watching microscopically for resistance to bending. Only undamaged hairs were used. While normal hairs formed evenly curved bends at the point of stress, with no sign of splitting, matted hairs bent sharply. Repeated bending of these produced splitting along the cortex (Text-fig. 1). It is clear, therefore, that matted hairs are less flexible than normal ones.



TEXT-FIG. 1. Matted awl hair showing a typical sharp bend and splitting at a point of repeated stress. Camera lucida drawing.



TEXT-FIG. 2A. Fresh sagittal section of normai skin to show the way the hairs flex down flat against the body. Camera lucida drawing.

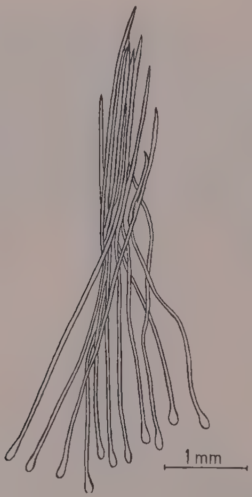
TEXT-FIG. 2B. Fresh sagittal section of matted skin to show the way hairs are erected owing to their inflexibility. Camera lucida drawing.

Comparisons of the orientations of hair shaft and follicle to the epidermis in normal and matted mice were made from sagittal sections of the skin. A fresh untreated segment of the skin was spread fur uppermost on a dry slide, a thick section was cut with a sharp blade, moistened, and arranged along the slide edge. By holding the slide vertically under the microscope and viewing by reflected light, the course of several follicles and hairs could be followed without further treatment (Text-fig. 2). The follicle angles of normal and matted hairs show no appreciable difference, but the emergent matted hairs do not curve back against the skin like the more flexible normal hairs.

Matted clumps of hair

The largest clumps are seen in the long hair of the back, usually when the coat is thin owing to hair loss. When the coat is thick, fewer hairs are found in each clump, the numerous small clumps giving a rough appearance to the fur.

When individual clumps were examined microscopically they were found to contain all the four hair-types described by Dry (1926). Counts of dorsal and ventral hair-samples gave similar proportions of guard-hairs, zigzags, awls, and auchenes to those for normal mice.



TEXT-FIG. 3. A matted clump of hairs, stuck together towards their tips. Camera lucida drawing.

Hairs in a clump tend to stick together distally (Text-fig. 3). Normally this adhesion results from the mouse grooming itself, for if the matted coat is brushed and combed free of clumps it is seen that clumping occurs again wherever the mouse licks its coat. This moistened paintbrush effect of surface tension can be simulated in a normal mouse by applying enough water and ruffling the fur, but it is not very noticeable during grooming as the coat is thick and flat against the skin. Observations on normal mice whose coats had been wetted and ruffled showed that clumps remained even after the hair had dried, and only disappeared when the mouse groomed itself, combing and flattening them with its tongue.

The matted mouse, however, cannot smooth down its fur because of the inflexibility of individual hairs; licking its coat therefore merely produces new clumps. The normal salivary mucus seems to help in holding the hairs together. This was suggested by the fact that separation of the hairs from matted clumps occurred after digestion with papain in saline, and to a lesser extent with plain saline, but not with distilled water even on shaking. Matted clumps of hair soaked in ether to remove fat remained stuck together on drying, so sebum is probably not involved. Fibres separated from each other microscopically generally had smooth edges, suggesting that frictional effects due to cortical damage is unimportant in keeping hair stuck together. This also followed from the discovery that if the coat of a normal mouse was well bleached with hydrogen peroxide (20 volumes), the hair became inflexible and erected, with marked clumping at once, before the brittle hairs could have been broken. The next coat generation following treatment was normal.

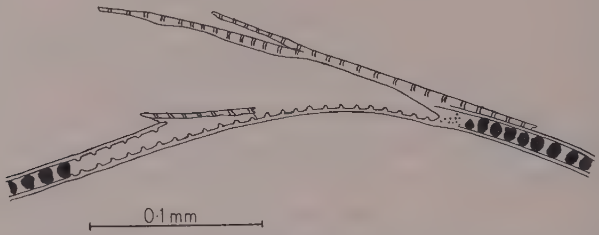
These facts, together with a detailed microscopic examination of matted clumps, suggest that the inflexibility of the hair is the underlying cause of clumping, while the general tendency of both matted and normal hairs to twist round each other when clumped, and the normal stickiness of the saliva help to keep hairs stuck together.

Tendency to baldness

The fact that the hair is inflexible and brittle suggests that breakage is the cause of baldness. Individual hairs were therefore plucked close to the skin and examined. Those from bald areas were often broken, but only minor splitting was seen where the coat was thick. Plucked matted hairs as often as normal retained the follicle bulbs, so tensile strength of fibres is good. It was found that when two very bald mice were placed in separate boxes without food-hoppers they regained a complete hair cover on growth of the next hair generation, although control matted sibs (with metal food-hoppers) stayed bald. Replacing the food-hopper in the experimental cage led to renewed baldness. Loss of dorsal hair is due therefore to friction between the coat and the metal food-hopper. Scratching the flanks may lead to small bald patches.

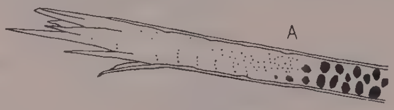
Individual hairs were examined in detail after passing through absolute alcohol, alcohol + xylene, and xylene (Dry, 1926), then mounting in Canada balsam or distrene. Serial transverse sections of hairs embedded in hard paraffin wax (m.p. 67° C.) were also cut at 10 μ with an ordinary microtome. A Hardy textile fibre microtome (Wildman, 1954) was also used. Measurements with a micrometer of the diameters of awls and zigzags showed no significant difference from normal.

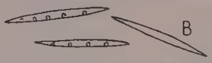
Intact matted hairs do not differ morphologically from normal hairs; the differences become noticeable as a result of mechanical stresses which damage matted hair but do not affect normal hair. During the first few days growth of a new matted coat no hair abnormalities are visible. Slightly later, however, splinters of cortical and cuticular keratin can be found partially broken off some fibres. In older hair-samples longitudinal splitting is often very severe; there may be complete breakage of the shaft (Text-figs. 4, 5A). This has been seen in all hair-types except sinus hairs; no particular region of the fibre is affected. Thus zigzags break as often in the middle of a segment as at the constrictions.



TEXT-FIG. 4. Matted zigzag hair showing typical longitudinal splitting, trichorrhexis. Camera lucida drawing.

At the time when splitting of hairs is first seen, certain regions along the shaft can be found where the accumulations of melanin pigment granules in medullary cells is dispersed or has disappeared entirely. It may be that bending of hairs which is insufficient to cause external breakage can sometimes disrupt medullary cells by compression at the bend, although this phenomenon was not seen in artificially bent hairs. Early in a hair generation hairs are fully pigmented.





TEXT-FIG. 5A. Matted awl hair broken across. The aspect of the jagged ends suggests that splitting occurs between the cortical cells rather than through them. Camera lucida drawing.

TEXT-FIG. 5B. Separated matted hair cortical cells. Camera lucida drawing. Same scale as Text-fig. 4.

The cortical cells of matted hair were examined after digestion for 48 hours in a solution of 2 per cent. papain plus 2 per cent. potassium bisulphite at 37° C. and grinding the digest with a soluble gritty salt, sodium citrate (Lennox, 1952). The spindle-shaped cells thus obtained (Text-fig. 5B) did not differ from those of normal mouse-hair cortex. To separate cells in both cases it was necessary to grind for about 10 minutes with pestle and mortar, so the cement substance does not appear to be weak. Nevertheless, the similarity between the jagged ends of broken matted hairs (Text-fig. 5A) and the spindle-shaped tips of the cortical cells suggests that splitting commonly occurs between cells rather than through them. This could be explained by the cement substance being normal in strength and the hair-cells being more rigid and inflexible than normal, so that on bending, splitting occurs at the place relatively weakest, between adjacent cells. Unlike matted, Naked (N / +) hairs examined were found to have cleanly broken ends and did not show much longitudinal splitting.

Matted and normal skin samples from young (2–3 weeks) and adult mice were fixed in aqueous Bouin's solution, sectioned sagittaly at 8 μ and stained in Ehrlich's haematoxylin and eosin. No hair breakages were found within matted follicle sheaths and the follicles themselves had well-formed bulbs. Sebaceous glands and other epidermal structures were normally developed. To check follicle density, skin fixed in aqueous Bouin's solution was macerated in warm 5 per cent. acetic acid for 4 hours and the underlying muscle dissected away. This method was modified from Dry (1926). After dehydration and clearing, the skin was mounted flat. Follicle numbers were counted under low power. Those for matted skin were of normal density even in bald areas.

Change in hair colour

The coat of a genotypically black-matted mouse tends to become russet in colour in old hairs. This change occurs even in the dark and affects the individual melanin granules which become translucent and brown (Plate, figs. D, E); hence it is not just the result of loss of melanin due to damage. The change in black melanin seems to result from the speeding up of a normal ageing process, for there is a tendency for some normal C57 black mice to become browner at the end of a coat generation, and this is also caused by an alteration in individual granules.

DISCUSSION

We have shown that the matted phenotype is due to a recessive gene which alters the mechanical properties of the hair. Hairs become inflexible and brittle, tending to split longitudinally and break off when bent. When compared with other hair genes in the mouse the action of matted shows clear similarities with that of Naked described by David (1932, 1934) and Fraser (1946), and Alopecia (Dickie, 1955), in that baldness results from the breakage of hairs above the skin and not from hairs falling out through malformed follicle end-bulbs, or from agenesis, but the threshold for breakage is much higher in matted than in these two mutants; environmental conditions therefore assume much greater importance in determining phenotypic appearance. This difference in threshold is well brought out by the time taken for baldness to develop: hair-loss is generally complete in a week in Naked N / + mice, but may not occur at all in matted. For this reason a sharp demarcation between hairy and bald areas is rarely seen in matted mice.

In Naked mice some of the first-formed hairs fail to penetrate the epidermis and remain coiled in the follicles. This phenomenon has not been found in matted hairs, which are brittle rather than soft. The matted gene agrees with

Naked N/+ in not affecting the sinus hairs, or general skin histology. Matted skin is not abnormally thickened or folded. In both Alopecia and Naked, scattered guard-hairs remain on otherwise bald areas, whereas in matted all types of hairs are equally affected and show breakage.

The matted type of hair-breakage with longitudinal splitting occurs as a rare abnormality in many mammals. In human trichorrhexis nodosa (Ormsby & Montgomery, 1954) the hairs are brittle and resemble matted in having good tensile strength. The severity of the defect varies, but typically affected hair remains short and looks rough as in matted mice. A change in hair colour has not been noticed. Although this abnormality has been recognized for well over a century its cause is still obscure, and while a similar complaint in horses is apparently an infection (Král & Novak, 1953) this seems not to be the case with the human disease. Hydrogen peroxide, which on normal mouse-hair gives a phenocopy of matted, can lead to trichorrhexis nodosa in human hair if used in excess.

The premature 'ageing' of melanin granules in matted is probably related to abnormal keratin formation, as the production of both is known to be interconnected. Thus Rauch (1952) has shown that in the rat avidin poisoning from a diet of raw egg albumen (the so-called biotin deficiency) results in dermatitis, hair brittleness and breakage, and abnormally light pigmentation. Tyrosine is a precursor of melanin and an important constituent of keratin and the blocking of certain enzyme syntheses is likely to affect both keratinization and melanin production. Tyrosinase is activated by copper, and Lerner & Fitzpatrick (1950) and others have shown that substances which remove free copper ions tend to reduce pigmentation by inactivation of this enzyme. Copper is also active at some stage in keratinization and the connexion is exemplified by the 'falling disease' in sheep fed on copper deficient pastures; Marston & Lee (1948) finding abnormal wool formation as well as more serious effects. Matted seems to differ from these conditions since melanin when first formed seems normal, but possibly it is unstable chemically. According to Baker & Andrews (1944) red melanin is an oxidation product of black melanin. When normal black mice are lightly bleached with hydrogen peroxide the russet colour produced resembles that seen in matted hair. Possibly the extensive colour change in old matted hair as well as the less-marked change in normal black hair result from oxidation of melanins differing in stability. Since all mice were kept in semi-darkness bleaching by light was not involved. The colour change is confined to the cortical and medullary melanin granules described by Russell (1949) and no change is seen in keratin in melanin-free places.

We can see then that the effect of the matted gene on the hair-fibres themselves, and on the pigment they contain both suggest that it interferes fundamentally with normal keratin formation, leading to what can be vaguely termed 'a defect in keratinization'. Studies on the keratin abnormality are described in another paper (Jarrett & Spearman, 1957).

SUMMARY

1. Matted (symbol *ma*) is a fully penetrant recessive mutant in the house-mouse which does not reduce fertility or viability under laboratory conditions.

2. It affects the coat, producing: (i) erection of the hairs, with the formation of matted clumps of hair, giving it an unkempt appearance; (ii) a variable amount of baldness, depending on areas of friction and resultant hair breakage; (iii) a colour change to russet which happens regularly towards the end of each hair regeneration in black hair.

3. The hairs are inflexible and brittle, tending to split longitudinally when bent. Medullary pigment granules are lost over short lengths of the hair, presumably because of breaks in the keratin. Tensile strength is normal.

4. Prolonged application of hydrogen peroxide to the normal mouse coat produces a phenocopy of matted which persists until the next hair generation is formed.

5. Similar conditions in mouse and man are discussed, and possible connexions between the abnormal keratinization and the change in hair colour.

ACKNOWLEDGEMENTS

We are grateful to Prof. H. Grüneberg, F.R.S., for the interest he has shown in this work. We also wish to thank Prof. E. Baldwin and Dr. N. Barnicot for advice and criticism. Mr. R. Lees kindly helped with photography and the manuscript was typed by Miss H. Bartels.

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EXPLANATION OF PLATE

FIG. A. Normal agouti mouse.

FIG. B. Matted agouti mouse showing the rough appearance of the coat with erection and clumping of the inflexible hair.

FIG. C. Matted black mouse with more pronounced clumping of hairs than in Fig. B. A bald patch is just visible on the back near the shoulders. This mouse had previously been completely bald on the back.

FIG. D. Pigment granules in normal black mouse-hair. The size of medullary pigment cells varies greatly. × 875. Cleaned Canada balsam preparation.

FIG. E. Pigment granules in an old matted black hair. Granules in the medullary cells are beginning to separate and individual granules have changed from black to brown in both medulla and cortex. × 875. Cleared Canada balsam preparation.

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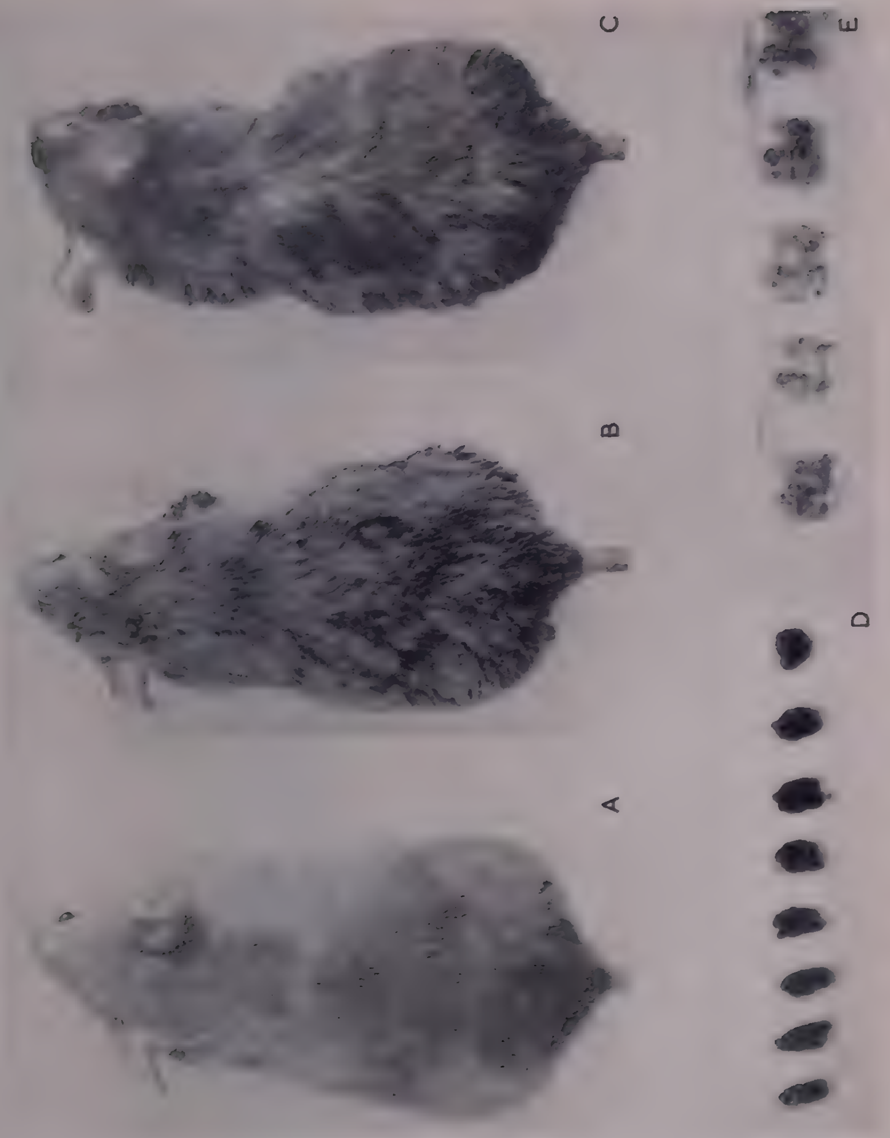

A. G. SEARLE *and* R. I. SPEARMAN

The Keratin Defect and Hair-cycle of a New Mutant (Matted) in the House-mouse

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WITH ONE PLATE

THE gene matted (symbol *ma*) reported by Searle & Spearman (1957) causes the body-hair of affected mice to be brittle and inflexible; this results in longitudinal splitting and breaking due to friction against the cage and other objects. In the present paper the keratin abnormality of the hair is studied. The defect in matted hairs is compared with that of Naked (N/+) mice (Grüneberg, 1952), and the hair-cycle in matted mice is described.

Keratins probably vary widely in composition, but attempts to study their chemistry have been only partially successful (Bach, 1952; Neurath & Bailey, 1954; Rothman, 1954). All keratins are rich in cystine, and it has been shown that oxidation of thiol SH groups to cystine S–S occurs during keratinization (Giroud & Bulliard, 1930; Hardy, 1952). The strength of keratin depends largely upon cystine linkages (Goddard & Michaelis, 1934), hydrogen bonds (Alexander, 1951), and polar (salt) linkages (Speakman & Townsend, 1938); these unite the long polypeptide fibre molecules. The amino-acid composition is also important in determining the properties of keratin, and Block & Vickery (1931) considered the relative proportions of basic amino-acids to be significant. Auber (1952) believed that lateral compression of the polypeptide fibrils by the turgid inner root sheath was an important factor in keratinization.

In the present study the keratin defect in the matted mutant was examined by ordinary staining methods and by fluorescence microscopy. These hairs were compared with normal mouse-hairs in which the keratin cross linkages had been artificially broken. The molecular structure of the hair has been examined by X-ray diffraction. Thiol and cystine sulphur have been studied, and the amino-acid content of the hairs estimated. The keratin defect is discussed in the light of these results.

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Histological examination

Sections of skin containing long-growing hair-follicles were treated by a modification of Auber's (1952) method. The tissue was bulked stained in Mayer's haemalum; sections were cut 6 μ thick and stained with 1 per cent. basic fuchsin and 0·1 per cent. picro-indigocarmine. The structure of the skin and hair-follicles in matted animals appeared normal, and we were unable, by this method, to detect any consistent abnormality in the keratinization of growing hairs within the follicle.

Fluorescence microscopy

Hairs of normal, matted, and Naked (N/+) mice, were treated with fluorochromes and examined microscopically by filtered ultraviolet rays. The most useful fluorochromes for this study were found to be acridine orange, thioflavine T, and rhodamine B. The hairs were plucked carefully in order to avoid trauma as far as possible. They were degreased in ether, dried, and then placed in 70 per cent. alcohol for 3 hours. After rinsing in distilled water, the hairs were immersed in a 0·1 per cent. aqueous solution of fluorochrome for 3 minutes. They were then rapidly rinsed in distilled water, dehydrated, cleared, and mounted in a non-fluorescing medium—DePeX. The apparatus employed was that described by Jarrett *et al.* (1956).

Hairs from normal mice were examined by this technique with acridine orange. These showed a green fluorescence; the contained melanin was dark and non-fluorescent. Very occasionally brilliant red areas were seen in the medulla. Patches of red fluorescence were found in a much higher proportion of, and were more extensive in, hairs of matted mice than in those of normal animals. The majority of matted hairs had one or more patches of red fluorescence (Plate, fig. A). By this means we were able to detect hairs from mutant mice as early as 9 days old before any breakage had occurred or the mice were abnormal to look at.

Normal hairs were cut, and then treated in the manner already described. It was then seen that the medulla at the cut end of the hair fluoresced red (Plate, fig. B). This fluorescence was indistinguishable from that occurring in matted hairs, and in a few of the normal hairs previously examined. This indicated that the normal fluorescence of the medulla when treated with acridine orange was red, but normal hair cuticle prevented the entrance of the fluorochrome. In the matted hair, and in cut normal hair, the fluorochrome penetrated into the medulla and caused it to fluoresce red. The cut tips of matted hairs also fluoresced in the same manner as normal hairs.

These findings led us to the conclusion that in matted hair the cuticle was defective. There was no evidence of any abnormality of the medulla, as the medullary fluorescence with acridine orange was the same in matted as in normal hairs. The abnormality occurred in patches in the cuticle, and these areas were shown by the fluorochrome entering the adjacent medulla and causing it to fluoresce.

The fluorochrome technique was repeated with rhodamine B and thioflavine T. It was seen that when cut normal hairs were treated with rhodamine B they fluoresced for a greater distance from the cut end than with acridine orange (Plate, fig. D). The colour of this fluorescence was salmon pink. It appeared that there was a better diffusion of this fluorochrome along the medulla. This was confirmed by treating matted hairs with rhodamine B; the patches of medullary fluorescence now extended up and down the hair shaft, and these often coalesced, causing the whole length of the medulla to fluoresce (Plate, fig. C). This increased penetration could be seen on macroscopical examination by ordinary light. Matted hairs treated with rhodamine B were stained a magenta colour, whereas normal uncut hairs retained their usual coloration. Thioflavine T had a similar degree of penetration to acridine orange; with this fluorochrome the medulla of matted hairs underlying the areas of defective cuticle fluoresced yellow. The cut ends of normal hairs and of cut matted hairs gave the same yellow fluorescence in the medulla.

These findings confirmed that in normal hair the cuticle prevents the medullary fluorescence from occurring unless it is artificially damaged as by cutting the hairs. The defective cuticle in matted hairs was also demonstrated by staining for 3 minutes with 0·1 aqueous solution of methylene blue and mounting in glycerine. The dye penetrated the cuticle of matted hairs but failed to do so in normal hairs.

The hairs of Naked (N / +) mice were examined with the same fluorochromes. In these hairs, in contrast to matted, there were areas of obvious anatomical defects. When treated with acridine orange these affected patches showed a red fluorescence of the cortex. The hair-shaft was often enlarged at these points, and the melanin granules were broken up (Plate, fig. E). Thus these hairs could easily be distinguished from matted hairs. The fluorescence with rhodamine B was salmon pink in the defective places, but the diffusion of this fluorochrome was not so good as in matted, and therefore the area of fluorescence were more limited. No alteration of the medullary fluorescence along the hair-shaft was detected by this method. The fluorescence obtained with thioflavine T confirmed the findings with acridine orange. The defective septa fluoresced yellow and green; the normal medullary fluorescence with this fluorochrome is yellow.

The effects of chemicals on the fluorescence of normal hair

The results obtained after treatment of plucked hairs with various chemicals are given in Table 1. Calcium thioglycollate which reduces S–S linkages (Goddard & Michaelis, 1934) increases normal cuticular permeability, and subsequent fluorescence with fluorochromes is similar to that of matted hairs. Hydrogen peroxide gives a quite different fluorescence from matted hairs, although treated fibres are similarly brittle and inflexible. Other reagents alter the normal hair fluorescence but have to be used in such strong concentration that fibre structure is grossly altered. It is possible that a deficiency of S–S linkages exists in the cuticle of affected areas of matted hairs.

TABLE 1

Fluorescence of chemically treated normal hairs with 0·1 per cent. acridine orange

	Fluorescence		
Chemical	*Cuticle and cortex*	*Cross septa and medulla*	*Notes*
1% Ca. thioglycollate . .	Intense green	80% red	Cystine S–S linkages reduced
Hydrogen peroxide (20 vols.)	Whole hair intense yellow-orange		Oxidation of cystine
Potassium metabisulphite (saturated solution)	Intense green-yellow	30% red	Breakage of cystine linkages
5 N NaOH	Intense green	30% red	Breakage of cystine and 'salt' linkages
5 N HCl	Light green	Little change from normal	Breakage of cystine and 'salt' linkages
Lithium bromide (saturated solutions)	Light green	30% red	Breakage of hydrogen bonds

Percentages refer to approximate proportion of hair fluorescing red

Sinus hairs and epidermal keratin

Sinus hairs of matted mice did not show an abnormal fluorescence as did the body-hairs. They gave the same green fluorescence as normal sinus-hairs. It was found, however, that when both normal and matted sinus-hairs were cut, the medulla did not fluoresce red when treated with acridine orange. If therefore there was a defect in the cuticle of these hairs the penetration of the fluorochrome into the medulla would not be shown by this method.

Skin from the back of normal and matted mice was fixed in 70 per cent. alcohol. Sections were cut and double-stained with acridine orange and primulin by the method described by Jarrett *et al.* (1956). There was no difference between the epidermal keratin of normal mouse skin and that of matted skin; in both the colour fluorescence was orange.

Physical and chemical examination of the hair keratin

No differences were noted between normal and matted hairs when these were examined by phase contrast microscopy and polariscopy. This suggests that the molecular arrangement in matted fibres is normal. Professor W. T. Astbury kindly examined the X-ray diffraction patterns of matted and normal mouse-hair. He found both molecular pictures to be of the α keratin type (Astbury, 1950), and there was no detectable alteration of the keratin in matted hairs. The chain molecular orientation in both was better than in most wool or human hair; this may lead to an increased tendency to longitudinal splitting in mouse hairs.

Hairs were examined for SH groups by the 'nitroprusside test' (Glick, 1949), but no free SH groups were demonstrated in either matted or normal fibres.

Quantitative estimations of total cystine were made in shaved samples of matted and normal mouse-hair by the colorimetric method developed by Lugg (1932 *a*, *b*) and by Shinohara (1937). The hair was degreased, washed, and dried: weighed amounts were hydrolysed by boiling 5 N hydrochloric acid. The cystine S–S was then reduced by sodium bisulphite to thiol SH, and these groups were estimated by the blue colour produced with active phosphotungstic acid reagent. (Some of these analyses were kindly performed by Dr. S. Blackburn.) Normal mouse-hair from the back contains about 2·4 per cent. cystine, but no appreciable thiol S; matted hair does not show any significant difference from the normal in composition.

Although the total cystine content is similar in matted and normal hairs, this does not exclude the possibility of an altered cystine distribution. A relative deficiency in the cuticle, which may render this structure more permeable, could be compensated by an increased amount in the medulla or in other areas of cuticle and cortex. The cystine content in wool-fibres is known to vary along their length, and the medulla is normally low in cystine (Speakman, 1955). An abnormal distribution, with a cystine deficiency in the cuticle in matted hairs, could on mechanical grounds explain their brittleness.

The amino-acid composition of matted and normal mouse-hair was examined by Dr. S. Blackburn. One-dimensional phenol-ammonia and two-dimensional collidine-phenol-ammonia chromatograms of keratin hydrolysates were prepared. No difference was found between the keratin composition of normal and matted hairs. The N-terminal amino-acids were not examined.

The effects of concentrated sodium hydroxide and hydrochloric acid on normal and matted hairs was examined. Both were less resistant to sodium hydroxide than to hydrochloric acid, but there was no appreciable difference between these hairs in their resistance to sodium hydroxide.

Urinary amino-acids and glucose

No glucose was detected in the urine of matted mice when tested with Benedict's reagent. Paper chromatograms of the urinary amino-acids of agouti matted and normal mice were prepared by the method of Harris & Searle (1953); in both, taurine was the only amino-acid detected. There was no evidence of cystinuria.

Histology of internal organs

Thyroids, adrenals, and livers from normal and matted mice were examined histologically after staining with haematoxylin and eosin. No abnormalities were seen.

Hair-cycle studies

The hair-cycle on the back was studied in normal and matted mice after lightly clipping the coat. There was no significant difference in the lengths of the first and second hair generations in these two groups; the third generation, however, was significantly shorter in matted mice (Table 2). This difference is possibly due to environmental factors rather than genetically determined. It is known that the mouse-hair-cycle is influenced by mechanical irritation (Borum, 1954). Owing to increased hair-loss in matted mice, the skin is less well protected against friction, and consequently this stimulation would result in a more rapid hair regeneration. This would explain the normal growth for the first two generations before the hair-loss is very great, while the third and subsequent regenerations were more rapid than in normal animals.

TABLE 2

Age in days on completion of first three dorsal coat generations (G_1, G_2, and G_3) in matted (ma/ma) *and normal* (+/ma) *mice. G_1 was completed in 9 days in every case*

Litter	*Mouse*	*Sex*	G_2	G_3
RR 59/60*c*	+/*ma*	♂	49	122
,,	,,	♀	46	107
,,	,,	♀	49	112
,,	,,	♀	49	112
,,	*ma/ma*	♀	42	72
RR 61/62*b*	+/*ma*	♂	41	96
,,	*ma/ma*	♂	38	59
,,	,,	♂	35	59
,,	,,	♂	35	59
,,	,,	♂	35	59
,,	,,	♀	38	64
,,	,,	♀	38	64
RR 55/56*c*	+/*ma*	♂	46	107
,,	*ma/ma*	♂	44	68

SUMMARY

1. The keratin abnormalities of the hair in matted and Naked (N/+) mice have been studied by fluorescence microscopy. Numerous areas of red fluorescence were noted in the medulla of intact matted hairs treated with acridine orange. This appearance differed from that of normal mouse-hairs, which showed only very occasional patches of red fluorescence except at their cut ends. The defect of matted hairs is thought to be in the cuticle; where this structure is permeable it allows the fluorochrome to penetrate into the medulla. Confirmatory results were obtained with other fluorochromes and methylene blue.

2. The permeability of normal mouse-hair-cuticle was increased greatly when the keratin was reduced with calcium thioglycollate.

3. Naked hairs unlike matted hairs showed gross anatomical defects of the

cross septa, and with acridine orange these fluoresced red in some areas and green in others.

4. Other histological methods failed to reveal any difference between matted and normal hair and skin.

5. Chemical and physical analyses of matted hairs showed no significant difference from normal; neither was there any evidence of abnormal amino-aciduria. It is suggested that an abnormal distribution of S–S linkages might upset the mechanical properties of matted hairs; the total composition remaining normal.

6. The hair-cycle of matted mice is described; this was found to be much shorter in the later hair generations. The cause of this more rapid regeneration is discussed.

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The Naked mice were kindly supplied by Dr. T. C. Carter of the Medical Research Council unit, Harwell.

Mrs. J. A. Hardy prepared the specimens for fluorescence microscopy, and Mr. A. Bligh helped in the production of the photo-fluoromicrographs.

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EXPLANATION OF PLATE

Fig. A. *Matted mouse-hair.* Fluorescence with acridine orange. The fluorochrome has penetrated into the medulla in one area, and this is fluorescing red. × 450.

Fig. B. *Normal mouse-hair cut transversely with scissors.* Fluorescence with acridine orange. The fluorochrome has entered the medulla for a short distance through the cut end, and caused it to fluoresce red. × 450.

Fig. C. *Matted mouse-hair.* Fluorescence with rhodamine B. Salmon coloured fluorescence extends along the whole length of the hair. × 100.

Fig. D. *Normal mouse-hair cut transversely.* Fluoresence with rhodamine B. The fluorochrome has entered the medulla for a greater distance than with acridine orange. × 200.

Fig. E. *Naked (N +) mouse-hair.* Fluorescence with acridine orange. The medulla is fluorescing red in some areas, and green in others; a gross anatomical defect can be seen in one of the hairs. × 200.

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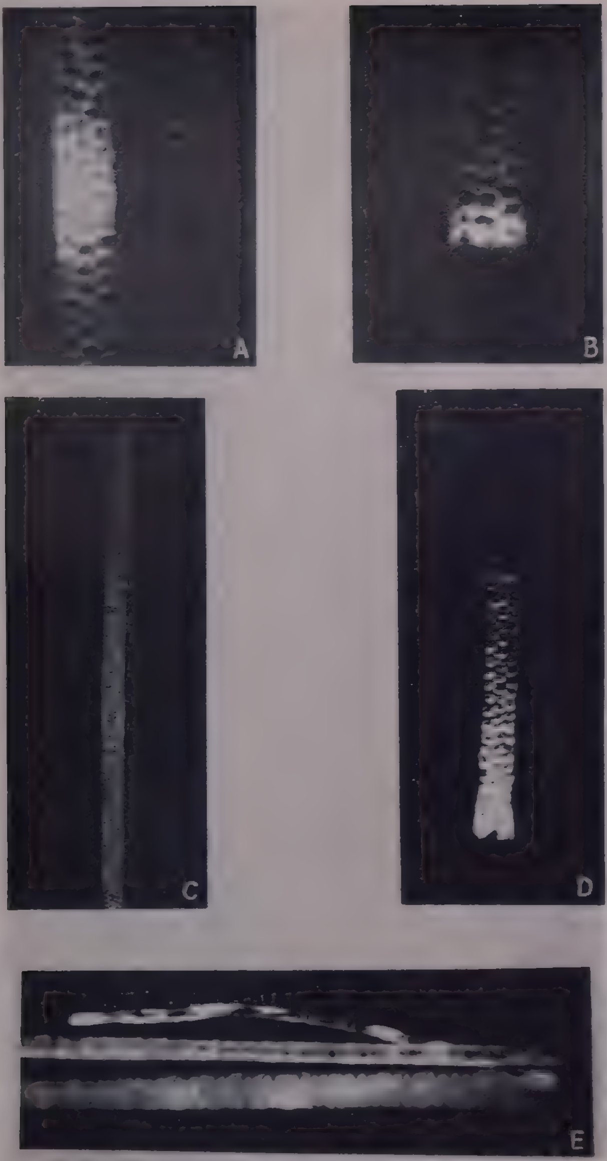

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